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Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome Trust

BBSRC

Cambridge Trust

Title: Developmental increase in cortical myelination and internodal length variability in the mouse neocortex

Authors: *E. PAMA, K. A. EVANS, P. HUMPHREYS, R. T. KÁRADÓTTIR;
Wellcome Trust - Med. Res. Council Cambridge Stem Cell Inst., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Myelin is essential for cognitive functioning and its loss or damage is associated with a number of neurological disorders and mental illnesses [1]. Alternations in myelination are increasingly being implicated as a mechanism for learning. Despite the importance of myelin, age-related myelination changes are poorly understood. Increases in white matter volume have been observed across several brain regions in childhood and adolescence, both in humans and other species[2,3,4]. However, while many studies investigate myelination in rodent models, the exact nature of developmental myelination changes in mice is still unclear. Importantly, differential myelination patterns have been found in distinct cortical layers within the mouse brain[5]. In addition to the overall myelination pattern, various other structural features such as the amount and length of internodes along axons may potentially affect conduction speed and change over time. In the peripheral nervous system, a functional relationship between internodal distance and conduction speed has been demonstrated and internodal length increases throughout development[6]. The question arises whether the same pattern occurs in the central nervous system (CNS). Therefore, we investigated myelin distribution and structure in the mouse brain neocortex at various time points during development (*p0-p118*). In addition to overall myelination we studied specific myelin features such as internodal length. We used immunohistochemistry to stain for myelin changes and by using high-resolution confocal imaging we were able to quantify cortical myelination and internodal distances. Our preliminary results indicate an increase in cortical myelination over time: a gradual increase in all cortical layers was observed, with no myelination present in the first week. While we indeed observed higher myelin density in deeper layers, our results suggest that the variability of internodal length (instead of overall internodal

length) continues to increase during the developing CNS. In summary, these results suggest that the development of complex neural circuits may be accompanied by characteristic, staged changes in myelination, highlighting the potential role of myelin in network synchronisation.

References

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Disclosures: E. Pama: None. **K.A. Evans:** None. **P. Humphreys:** None. **R.T. Káradóttir:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.02/A2

Topic: B.12. Glial Mechanisms

Support: Supported by MDA

Target ALS

Title: Characterization of an *In vivo* tool to study oligodendrocyte metabolic support to neurons

Authors: *T. PHILIPS, B. M. MORRISON, J. D. ROTHSTEIN;
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Abstract: Numerous studies have shown that neuronal metabolism is highly dependent on the metabolic support they receive from their neighboring glial cells. Astrocytes and oligodendrocytes are now well established as cells important for providing metabolic support to neurons. One particular pathway involved is mediated by the monocarboxylate transporters (MCTs) that transport lactate, pyruvate and ketone bodies across the plasma membrane, to be taken up by neurons as important alternative metabolic substrates. We have previously shown that one of these MCTs, MCT1, is strongly expressed by oligodendrocytes. Local delivery of

MCT1-shRNAs targeting oligodendrocytes leads to neuron death and axon degeneration, suggesting oligodendrocytes provide essential metabolic support to neurons.
In the current study we have developed new tools to characterize MCT protein expression in the CNS and their metabolic supportive role to neurons. Using newly generated, highly specific antibodies for MCT1 as well as using a MCT1-GFP expressing tag-in reporter mouse, we found that in addition to oligodendrocytes, MCT1 was expressed in astrocytes, endothelial cells and ependymal cells throughout both human and mouse CNS regions. However, antibody expression cannot define the relative quantitative role for this transporter in the various glial and endothelial cells. In order to better understand the MCT1 mediated metabolic supportive role in oligodendrocytes, we developed a conditional knockout mouse for MCT1, in which MCT1 exon2 is inserted between two loxP sites. When crossed with oligodendrocyte Mogi-Cre mice, homozygous MogiCre::MCT1loxP/loxP mice showed a >90% reduction of MCT1 protein in myelin preps of CNS tissue. Interestingly, these mice did not develop gross pathological or behavior abnormalities up to one year of age. On the other hand, mice which were knockout (KO) for MCT1 on one allele and had a complete knockout specifically in oligodendrocytes on the other allele (MogiCre::MCT1loxP/KO) developed strong pathology (astrogliosis, degeneration) at six months of age in different brain regions. Heterozygous MCT1 KO control mice did not show any behavior or pathological abnormalities. This data suggests that oligodendrocyte loss of MCT1 under normal physiological conditions is insufficient to cause severe neuronal injury but that loss of MCT1 in other cell types, in addition to oligodendrocytes, contributes to a highly significant brain pathology. We are currently assessing the behavioral consequences associated with this pathology as well as trying to understand how other cell types like astrocytes and endothelial cells are involved in this injury response.

Disclosures: T. Philips: None. **B.M. Morrison:** None. **J.D. Rothstein:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.03/A3

Topic: B.12. Glial Mechanisms

Title: Vesicular trafficking in Schwann cell development and myelination

Authors: *B. ABDELMESIH, C. EYERMANN, C. MELENDEZ-VASQUEZ;
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Abstract: Myelination in the peripheral nervous system is carried out by Schwann cells (SC), which enwrap and insulate axons facilitating rapid transmission of nerve impulses. Non-muscle myosin II (NMII), an actin-binding motor protein, is a key regulator of cytoskeleton dynamics necessary for interactions between SC and axons and normal myelin formation. NMII activity is regulated by the phosphorylation of its regulatory light chain (MLC). Previous work from our laboratory indicated that one of the kinases that phosphorylates MLC, myosin light chain kinase (MLCK) is required for normal SC myelination. Knockdown of MLCK in SC results in prominent accumulation of large vesicles and increased autophagic and lysosomal protein expression suggesting that MLCK activity is necessary for proper protein trafficking. We now show Rab7+ vesicles accumulate in SC in the absence of MLCK activity. Rab7 is a GTPase that regulates late endosomal trafficking and lysosomal biogenesis. We find this effect only in the presence of MLCK inhibitor and not with inhibitors of NMII phosphorylation or motor activity. Furthermore, we show significantly increased co-localization between Rab7 and Rab9. Rab9 is a GTPase important for transporting lysosomal hydrolases between the trans golgi network and late endosomes. Inhibition of MLCK and accumulation of Rab7-Rab9+ vesicles may suggest that lysosomal enzymes are mislocalized. Currently, we are investigating the mechanism by which MLCK activity is required for proper Rab7 trafficking, and how Rab7 function may be required for myelin proteins to be delivered to the plasma membrane to achieve normal myelination.

Disclosures: B. Abdelmesih: None. **C. Eyermann:** None. **C. Melendez-Vasquez:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.04/A4

Topic: B.12. Glial Mechanisms

Support: Swedish Research Council

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Swedish Brain Foundation

Swedish Society of Medicine

Åke Wiberg foundation

Clas Groschinsky foundation

Petrus och Augusta Hedlunds foundation

Title: Investigating oligodendrocyte axonal preference in the mammalian central nervous system. A whole-tissue high-resolution approach.

Authors: *E. M. FLORIDDIA, C. BELLARDITA, P. LÖW, O. KIEHN, G. CASTELO-BRANCO;
Karolinska Institutet, Stockholm, Sweden

Abstract: Oligodendrocytes (OLs) are the myelinating cells in the central nervous system (CNS). They insulate axons affecting the conduction speed of action potentials, making OLs very important actors in neurological functions and disorders. Single-cell RNA-Seq studies performed in our laboratory show that the CNS comprises 12 subpopulations belonging to the OL lineage, spanning from oligodendrocyte precursor cells (OPCs) to mature OLs (MOLs). These data also highlight transcriptional differences among myelinating OL subpopulations. However, the impact of OL transcriptional heterogeneity on their functionality is unclear. One important and unanswered functional question is how OLs decide what neurons to myelinate. Also whether certain OL subpopulations specifically myelinate only certain type of axons is unexplored. To address these questions, we use whole-tissue high-resolution imaging (CLARITY method). This approach allows us to acquire sufficiently detailed and large 3D images to analyze the intricate and heterogeneous morphologies of OLs and interactions with neurons in the intact cortex and spinal cord. We will fate map OPCs, label OL subpopulations with newly found-specific markers, and describe their interaction with different kind of neurons via CLARITY-optimized light-sheet (COLM) or confocal microscopy. We will discriminate neurons based on location (white and grey matter), morphology (long- or short-distant projecting), and activity (excitatory or inhibitory). Therefore, we will be able to correlate OL and neuronal types at high resolution and for the entirety of the OL and neuronal processes extension. We will analyze the acquired images to obtain 3D visualizations of OL morphologies, OLs-neurons interactions, and quantification of internodes number and length. Importantly, our approach does not rely on the assumption that the tissue is homogeneous, as 3D reconstruction based on tissue sections does. In conclusion, we aim to unveil the OL axonal preference in the mammalian CNS, shedding light on the fine-tuned cellular dynamics and functional heterogeneity of the OL lineage.

Disclosures: **E.M. Floriddia:** None. **C. Bellardita:** None. **P. Löw:** None. **O. Kiehn:** None. **G. Castelo-Branco:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Title: Preservation of Schwann cell identity *In vitro* through bone morphogenetic protein signaling

Authors: *Y.-S. CHAN, Y. P. TSUI, D. K. Y. SHUM;
Sch. of Biomedic. Sci., Fac. Med., Univ. Hong Kong, Hong Kong, China

Abstract: In the injured nerve environment, Schwann cells switch on an axon-supportive program that improves nerve regeneration and functional recovery. We therefore devised a protocol to derive Schwann cells from bone marrow stromal cells (Shea et al, Exp Neurol, 2010). In standard cultures of Schwann cells extracted from sciatic nerves of neonatal rats, we serendipitously observed expression of Olig2, the obligate transcription factor for oligodendrogenesis, as of 25 DIV; these Olig2-positive cells showed polydendrocytic morphology reminiscent of oligodendrocyte precursors. Increase in Olig2-positive cells was accompanied by decline in p75-immunopositivity among continuing cultures of the Schwann cells. We then hypothesized that the peripheral nerve environment harbours factors that preserve Schwann cell identity. In support of this, co-cultures of 25-DIV Schwann cells with neurons purified from dorsal root ganglia (DRG, E15) led to decline in incidence of Olig2-positive oligodendrocyte precursor cells. Our search for factors that suppress Olig2 expression found BMP4-immunoreactivity localized to the DRG neurons. Supplementation of BMP4 to 25-DIV Schwann cell cultures suppressed Olig2 expression but preserved p75 expression and the spindle morphology of Schwann cells. Preservation of Schwann cell identity *in vitro* therefore requires signalling cues, including BMP4, presented by peripheral neurons.

Disclosures: Y. Chan: None. **Y.P. Tsui:** None. **D.K.Y. Shum:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant NS084326

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Title: Human Schwann cell senescence is not prevented by ectopic expression of human telomerase reverse transcriptase

Authors: *N. D. ANDERSEN[1], B. KUO[1], G. PIÑERO[1], K. RAVELO[1], P. RAI[2], P. MONJE[1]; [1]The Miami Project to Cure Paralysis, Dept. of Neurolog. Surgery, [2]Dept. of Med., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Isolated human Schwann cells (hSCs) typically become senescent and unresponsive to mitogenic factors with continued in vitro expansion. Data from our lab has shown that adult nerve-derived hSCs, in contrast to rodent (rat) SCs, inevitably stop proliferating and acquire a senescent phenotype characterized by high levels of senescence-associated (SA)-β galactosidase activity and morphological changes that include cell enlargement and appearance of multi-nucleated cells. As opposed to rodent SCs, hSC cultures consist of mixed populations of proliferating cells, senescent cells and cells at different stages of differentiation regardless of the nerve of origin and other donor-specific factors. RNA-seq analysis of representative cultures of hSCs did not reveal the presence of telomerase reverse transcriptase (TERT) mRNA while other TERT-related genes (e.g. TERF1, telomeric repeat binding factor, and TEP1, telomerase-associated protein) were well-represented in the hSC transcriptome. In an attempt to overcome senescence, we used retroviral vectors and antibiotic selection to generate hSC lines ectopically expressing human (h)-TERT. For these experiments, highly proliferative, non-senescent, early passage hSC cultures were stably transduced with the retroviruses h-TERT-hygro or h-TERT-puro, each encoding the h-TERT gene along with hygromycin or puromycin resistance genes, respectively. Transduced hSC cultures from three different donors were selected and subjected to three rounds of expansion in medium containing chemical mitogens. Subsequently, the cultures were analyzed for their rate of proliferation by means of EdU incorporation assays and the acquisition of senescence by means of SA-β galactosidase activity assays in each round. We

found that whereas ectopic h-TERT expression extended the lifespan of cultured hSCs when compared to non-infected or GFP-expressing cells, it was not sufficient to confer immortalization and overcome senescence. In sum, our results suggest that progression of the hSCs to a senescent state likely is stress-induced rather than dependent on replication-associated telomere shortening.

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Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH1R21 Grant HD085490-01

Shriners Hospitals for Children

Title: Role of neural stem factor sox2 in postnatal oligodendrocyte development

Authors: ***S. ZHANG**[1,2], C. CREATO[1], E. HAMMOND[1], D. PLEASURE[1,2], J. XU[1], L. SONG[1,2], F. GUO[1,2];
[1]Inst. For Pediatric Regenerative Medicine, Sh, Sacramento, CA; [2]Departments of Neurology,School of Medicine, Univ. of California, Davis, Sacramento, CA

Abstract: Sox2, a SoxB1 transcription factor, plays an important role in maintaining the stemness of neural stem cells (NSCs) in both embryonic and adult ages in the central nervous system (CNS). It is generally thought that Sox2 is downregulatedor absent in the progenies derived from NSCs. Our previous study reports that Sox2 expression is maintained in the adult quiescent astrocytes (Guo et al., 2011 J Neurosci). Here, we showed a dynamic expression pattern of Sox2 in the oligodendroglial lineage cells.Sox2 is expressed at low level in virtually all oligodendroglial progenitor cells (OPCs) at both early postnatal and adult CNS, significantly upregulated in premyelinating oligodendrocytes (OLs) and downregulated in postmyelinating OLs after the completion of CNS myelination. Based on these observation, we hypothesize that Sox2 may play stage-dependent roles (**CNS developmental stages and oligodendrocyte developmental stages**) during postnatal oligodendrocyte development. Using inducible Cre-LoxP conditional knockout (cKO) system to ablate Sox2 in early postnatal OPCs, we

demonstrate Sox2 is required for OPC proliferation and dispensable for maintaining OPC survival in vivo during postnatal CNS development. The proliferation of OPCs (evidenced by Ki67 and EdU labeling) was significantly reduced in Sox2 cKO CNS compared to that in Sox2 wild type (WT) CNS whereas OPC apoptosis (evidenced by active caspase 3 and TUNEL labeling) was unaffected. Consistent with a reduction of OPC density, we demonstrated that the density of CC1+ differentiated OLs, including TCF7l2+ premyelinating OLs (Hammond et al., 2015, J Neurosci) was significantly decreased in Sox2 cKO CNS. Previous study has reported that Sox2 is dispensable for the proliferation of embryonic OPCs (Hoffman et al., 2014, Development). Our study suggests that Sox2 function in oligodendroglial lineage cells in a developmental stage-dependent (**embryonic versus postnatal**) manner. We are currently using genetic cKO system to specifically ablate Sox2 in oligodendroglial lineage cells downstream of OPCs and to determine the developmental stage-dependent role (**OPCs versus OLs**) along the linage progression of oligodendrocytes. Our study is likely to reveal a novel role of neural stem factor Sox2 in the development of oligodendroglial lineage cells.

Disclosures: S. Zhang: None. **C. Creato:** None. **E. Hammond:** None. **D. Pleasure:** None. **J. Xu:** None. **L. Song:** None. **F. Guo:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

Support: MOST 103-2314-B-006-007-MY3, Taiwan

MOST 104-2811-B-006-035, Taiwan

Title: Regulation of oligodendrocyte differentiation by the control of B-cell CLL lymphoma 11B expression

Authors: C.-Y. WANG[1], K.-M. FANG[2], C.-H. HO[2], *S.-F. TZENG[3];
[1]Life Sci., [2]Natl. Cheng Kung Univ., Tainan, Taiwan; [3]Natl. Cheng Kung Univ., Tainan City, Taiwan

Abstract: B-cell CLL/lymphoma 11B (Bcl11b), known as CTIP2, is a C2H2 zinc finger transcriptional factor. It functions as a haploinsufficient tumor suppressor in T cells, and involves in DNA damage and cell apoptosis. Our previous study has indicated that the enriched

expression of Bcl11b was detected in tumorigenic C6 glioma cells. In the present study, we found that Bcl11b was highly expressed in glial progenitors (GPCs). The biological roles in GPCs and glioma cells were further determined by lentivirus-mediated knockdown (KD) approach. The results showed that inhibition of Bcl11b expression in GPCs not only halted their proliferation, but also reduced stemness-related gene expression in the growth condition. However, the expression of Notch signaling genes was not affected by Bcl11b KD. When maintained in the differentiation medium for 5 days, GPCs with Bcl11b KD differentiated into mature CNPase^{+} oligodendrocytes compared to those observed in mock culture. Moreover, through the co-culture of GPCs with hippocampal neurons, the processes of oligodendrocytes derived from Bcl11b-KD GPCs were highly co-localized with hippocampal fibers when compared to the control group. In addition, the in vivo study using lysolecithin-induced demyelinating animal model indicated that Bcl11b-KD GPCs injected to the lesioned site of the white matter gave rise to more MBP^{+}-oligodendrocytes than the control group had. Altogether, our results demonstrate that Bcl11b is generally required to maintain the cell proliferation of glial progenitors; however, the downregulation of Bcl11b expression might accelerate oligodendrocytic maturation.

Disclosures: **C. Wang:** None. **K. Fang:** None. **C. Ho:** None. **S. Tzeng:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

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NIH R01NS062796

NIH R01AR064551

Title: Zeb2 recruits Hdac-NuRD to inhibit Notch and controls Schwann cell differentiation and remyelination

Authors: *L. WU[1], J. WANG[1], J. R. CHAN[2], M. JANKOWSKI[1], D. HUYLEBROECK[3], Q. LU, 45229[1];
[1]CCHMC, Cincinnati, OH; [2]UCSF, San Francisco, CA; [3]KU Leuven Dept. of Develop. & Regeneration, Leuven, Belgium

Abstract: The mechanisms that coordinate and balance a complex network of opposing regulators to control Schwann cell (SC) differentiation remain elusive. Here we demonstrate that zinc-finger E-box binding-homeobox 2 (Zeb2/Sip1) transcription factor is a critical intrinsic timer that controls the onset of Schwann cell (SC) differentiation by recruiting HDAC1/2-NuRD co-repressor complexes. *Zeb2* deletion arrests SCs at an undifferentiated state during peripheral nerve development and inhibits remyelination after injury. Zeb2 antagonizes inhibitory effectors including Notch and Sox2. Importantly, genome-wide transcriptome analysis reveals a Zeb2 target gene, encoding the Notch effector Hey2, as a potent inhibitor for SC differentiation. Strikingly, a genetic Zeb2 variant, which is associated with Mowat-Wilson syndrome, disrupts the interaction with HDAC1/2-NuRD and abolishes Zeb2 activity for SC differentiation. Therefore, Zeb2 controls SC maturation by recruiting HDAC1/2-NuRD complexes and inhibiting a novel Notch-Hey2 signaling axis, pointing to the critical role of HDAC1/2-NuRD activity in peripheral neuropathies caused by *ZEB2* mutations.

Disclosures: L. Wu: None. **J. Wang:** None. **J.R. Chan:** None. **M. Jankowski:** None. **D. Huylebroeck:** None. **Q. Lu:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.10/A10

Topic: B.12. Glial Mechanisms

Support: R01 NS057456

Title: Ionotropic glutamate receptor-triggered cell signaling in Schwann cells

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Abstract: We previously demonstrated that rat Schwann cells (SCs) express the N-methyl-D-aspartate receptor (NMDA-R). In this study, we performed PCR to identify specific subunits of

ionotropic glutamate receptors in cultured rat SCs. The NMDA-R-associated subunits: NR1, NR2B, NR2C, NR2D, and NR3B were present at readily detected levels in SCs. We also detected the AMPA Receptor subunits: GluA1, GluA2, and GluA3, and the Kainate Receptor subunits: GluK2, GluK3, GluK4, and GluK5. To test whether SC ionotropic glutamate receptors trigger cell-signaling, cells were exposed to glutamate for 15 min. Protein phosphorylation was assessed using the R&D Systems Phospho-protein Proteome Profiler. Substantial phosphorylation events were noted, including ERK1/2 and c-Jun. The PI3K-Akt pathway was prominently activated, including the downstream kinase, p70 S6 kinase. Activation of Akt may have been supported by mTORC2. The transcription factor, CREB was phosphorylated as well. Proteome Profiler experiments were performed using human SCs and a strikingly similar signaling response to glutamate was noted.
Glutamate did not induce SC death at concentrations up to 1.0 mM. In Transwell cell migration experiments, glutamate significantly promoted SC migration ($p<0.05$). The effects of glutamate on SC migration were enhanced by adding glycine (50 nM). To determine which ionotropic glutamate receptor is responsible for the activity of glutamate in SC signaling, we studied ERK1/2 phosphorylation, as a representative cell-signaling event, by immunoblot analysis. ERK1/2 phosphorylation was substantially inhibited, by more than 50%, by MK801 or by silencing expression of the NR1 subunit of the NMDA-R. MK801 also inhibited SC migration induced by glutamate. Collectively, these results suggest that the NMDA-R plays a prominent role in eliciting SC responses to glutamate and may participate in SC trans-differentiation that is essential for nerve repair.

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Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.11/A11

Topic: B.12. Glial Mechanisms

Title: Impact of Mtmr2 knockdown in Schwann cells

Authors: J. KIM, R. DOBROWOLSKI, *H. A. KIM;
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Abstract: Charcot-Marie-Tooth 4B1 is a form of inherited peripheral neuropathy that affect myelin in the peripheral nervous system (PNS). In human, the mutations affect the gene for

myotubularin-related protein 2 (Mtmr2) in an autosomal recessive manner. Genetic knockdown of Mtmr2 in mice recapitulates several aspects of CMT4B1, including reduced nerve conduction, loss of myelinated axons and myelin out-folding. More important, a previous study has shown that loss of Mtmr2 in Schwann cells, not neurons, contributes to the PNS myelin abnormalities, indicating the importance of the Schwann cell intrinsic function of Mtmr2 in normal development of the PNS myelin.
Mtmr2 is a phosphoinositide 3-phosphatase that uses PI(3,5)P2 and PI(3)P to generate PI(5)P and PI, respectively. A previous study has shown that Mtmr2 loss results in accumulation of PI(3,5)P2 in cells, indicating that the Mtmr2-associated myelin abnormalities may be due to dysregulation of the biological function related to PI(3,5)P2. PI(3,5)P2 is found within the endomembrane system, specifically within membranes of late endosomes and lysosomes. Therefore, it is possible that aberrant regulation of the endo-lysosome system may contribute to the myelin dysregulation in Schwann cells.
To investigate the effects of Mtmr2 loss in Schwann cells, we used lentivirus-mediated shRNA transduction to generate Schwann cell cultures in which Mtrm2 protein levels were knocked-down (KD) by 70-80%. While the morphology of Mtmr2 KD Schwann cells was indistinguishable from control cells under non-differentiation condition, upon differentiation by cAMP, the KD Schwann cells exhibited expanded membrane protrusions and enlarged cell bodies. A similar change in cell morphology was also evident when the Schwann cells were placed in contact with DRG neurons. Western blot analysis on differentiated KD Schwann cells showed a marked decrease in Krox 20, a pro-myelinating transcription factor, but no difference in myelin protein expression compared to the control cells. In the KD cells, we also observed an increase in the inhibition (phosphorylation) of Ulk1, a kinase that promotes autophagosome biogenesis. Mtmr2 KD Schwann cells expressed lower levels of p62 compared to the control, which may indicate a defect in autophagosome formation. Interestingly, autophagic flux appears to occur normally in the KD cells, indicating the presence of functional lysosomes. Altogether, our data show that loss of Mtmr2 in Schwann cells impacts cell morphology, Schwann cell differentiation and autophagy.

Disclosures: **J. Kim:** None. **R. Dobrowolski:** None. **H.A. Kim:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.12/A12

Topic: B.12. Glial Mechanisms

Support: P20 GM103554

P20 GM110767

Title: Schwann cell population responses to nerve stimulation

Authors: *T. W. GOULD, D. J. HEREDIA, G. W. HENNIG;
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Abstract: Terminal/perisynaptic Schwann cells at the adult neuromuscular junction (NMJ) respond to and regulate neurotransmission. During the early postnatal period of developmental synapse elimination, Schwann cells differentially decode competing neural inputs and help drive the turnover of these inputs by competing with them for space on the postsynaptic muscle fiber. During the embryonic period of synapse formation, Schwann cells maintain nascent synapses by antagonizing the effects of neural activity. Therefore, we have been characterizing the effects of peripheral motor nerve stimulation on Schwann cell calcium responses at the mouse neonatal neuromuscular junction (NMJ) using genetically encoded calcium indicators. We found that in the postnatal day 0 (P0) diaphragm muscle, phrenic nerve stimulation at a frequency and duration that causes a rundown of neurotransmitter release resulted in a robust accumulation of calcium by Schwann cells in the diaphragm. Interestingly, low-frequency nerve stimulation also induced calcium responses in Schwann cells at P0. The intensity, rise-to-peak intensity, duration, onset, and temporal heterogeneity of these Schwann cell calcium responses varied according to stimulation frequency and duration. We also examined the spatial pattern of Schwann cell responses to nerve stimulation over time. Whereas all Schwann cells at the NMJ and along pre-terminal axon branches responded to nerve stimulation at P0, only those at the NMJ responded at P7 and older. In contrast, Schwann cells along major phrenic nerve branches, as well as some but not all Schwann cells along terminal branches and at nascent NMJs, responded to nerve stimulation at early embryonic ages (E14-E15). Consistent with a previous report, the calcium accumulation within all Schwann cells during the neonatal period was completely blocked by a specific antagonist to P2YR1 receptors. These studies suggest that Schwann cells respond differentially to nerve stimulation at early ages. Further work is required to understand the mechanism underlying these differential responses.

Disclosures: T.W. Gould: None. **D.J. Heredia:** None. **G.W. Hennig:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.13/A13

Topic: A.03. Stem Cells and Reprogramming

Title: *In vitro* modeling of Canavan´s disease using human induced pluripotent stem cells

Authors: ***J. SAAL**[1,2], J. FISCHER[2], V. KAPS[2], M. MIZHOROVA[2], M. ECKHARD[3], J. O. SASS[4], M. KARUS[2], O. BRÜSTLE[2];
[2]Inst. of Reconstructive Neurobio., [3]Inst. for Biochem. and Mol. Biol., [1]Univ. of Bonn, Bonn, Germany; [4]Dept. of Natural Sci., Bonn Rhein-Sieg Univ. of Applied Sci., Rheinbach, Germany

Abstract: Canavans Disease (CD) is a fatal autosomal-recessive leukodystrophy primarily affecting children under one year of age. It is caused by mutations in the aspartoacylase gene (ASPA). The enzyme encoded by this gene cleaves N-acetylaspartate (NAA), yielding aspartate and acetate. The pathology is characterized by massive vacuolization of the brain and loss of myelin. The pathogenetic mechanisms underlying these phenotypes remain elusive. Here we employ human induced pluripotent stem cells (iPSCs) derived from four CD patients to model aspects of this disease in cell culture. Specifically, we reprogrammed patient fibroblasts using Sendai viral vectors encoding Sox2, Oct3/4, Klf4 and c-Myc. Resulting CD-hiPSCs were fully validated for virus inactivation, pluripotency marker expression (Tra1-60, Tra1-81, SSEA4) and their ability to differentiate into all three germ layers *in vitro* and *in vivo*. CD-hiPSCs were then differentiated into stably self-renewing radial glia-like neural progenitor cells (RGL-NPCs) according to a recently established protocol (Gorris et al., Glia 63:2152-67, 2015). These RGL-NPCs express typical radial glia markers including BLBP, CD133, Nestin, Sox2, Sox9 and Pax6 and were used to generate CD-specific βIII-tubulin-/MAP2-positive neurons, O4-/MBP-positive oligodendrocytes and GFAP-positive astrocytes for phenotypic analysis. Detailed immunofluorescence and electron microscopic analyses revealed that CD-specific astrocytes contain significantly larger mitochondria with abnormally structured cristae. Specifically, Feret's diameters of mitochondria in CD-specific astrocytes were enlarged to 1.26 ± 0.06 μm (controls from healthy donors: 0.83 ± 0.05 μm; $p<0.005$). These observations correspond well with previous reports on mitochondrial aberrations in astrocytes of CD autopsy specimens, indicating that this pathological hallmark can be modeled in an in vitro iPSC system. We expect this model to provide further information on disease-specific metabolic alterations, potential pathological changes in neurons and oligodendrocytes, and the role of cell-autonomous and non cell-autonomous pathomechanisms underlying this disease.

Disclosures: **J. Saal:** None. **J. Fischer:** None. **V. Kaps:** None. **M. Mizhorova:** None. **M. Eckhard:** None. **J.O. Sass:** None. **M. Karus:** None. **O. Brüstle:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.14/B1

Topic: A.01. Neurogenesis and Gliogenesis

Support: Australian Research Council Specific Initiative in Stem Cell Science (Stem Cells Australia)

Title: Efficient pharmacogenetic ablation of oligodendrocyte progenitor cells (NG2 glia) in mice

Authors: *T. D. MERSON[1,2], B. H. A. CHUANG[2], T. J. KILPATRICK[3], Y. L. XING[2]; [1]Florey Dept of Neurosci. and Mental Hlth., University Of Melbourne, Australia; [2]Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia; [3]Dept of Anat. and Neurosci., Univ. of Melbourne, Parkville, Australia

Abstract: Remyelination of the central nervous system is primarily achieved by generating new myelin-forming oligodendrocytes, which can be derived from both parenchymal oligodendrocyte progenitor cells (OPCs/NG2 glia) and neural precursor cells (NPCs) that reside in the adult subventricular zone (SVZ). Our previous studies have demonstrated that NPCs are major contributors to oligodendrogenesis and remyelination following cuprizone-induced demyelination, but on a regionally restricted basis. We demonstrated that NPC-derived oligodendrocytes predominantly remyelinate regions of the demyelinated corpus callosum that are adjacent to the SVZ whereas OPC-derived oligodendrocytes occupy regions of the remyelinated corpus callosum that are distal to the SVZ. To explore whether the regionally restricted migration of NPCs reflects spatial competition with OPCs for oligodendrogenesis and remyelination, an approach to conditionally ablate OPCs while sparing the NPC population is required. Here we describe a highly efficient methodology to conditionally ablate parenchymal OPCs by comprising both genetic and pharmacological techniques. This approach could be adopted to examine the influence of OPC ablation upon the regenerative capacity and function of NPC-derived oligodendrocytes in demyelinating disease. In addition, the model provides a long sought after methodology to investigate the function of OPCs/NG2 glia in the normal healthy brain.

Disclosures: **T.D. Merson:** None. **B.H.A. Chuang:** None. **T.J. Kilpatrick:** None. **Y.L. Xing:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.15/B2

Topic: A.01. Neurogenesis and Gliogenesis

Support: Swiss National Science Foundation

Christopher and Dana Reeve Foundation

European Research Council (ERC) advanced grant

Title: Regulatory function of nogo-a in oligodendrocyte differentiation

Authors: *M. A. MAIBACH, F. MÜLLER, O. WEINMANN, M. E. SCHWAB;
Health, Sci. and Technol., ETH Zürich, Zürich, Switzerland

Abstract: The membrane protein Nogo-A was discovered in 1988 as a myelin-associated inhibitor of neurite outgrowth and regeneration in the adult central nervous system (CNS). In the following decades, Nogo-A emerged as a regulator of various other developmental and plastic processes such as synapse formation and neuronal migration. While Nogo-A is expressed by subtypes of neurons early in development, Nogo-A is mainly expressed throughout the oligodendroglial lineage in the postnatal CNS. Nogo-A signals via two extracellular regions termed Nogo-66 and Nogo-A-Δ20. The Nogo-66 signals are transduced by a Nogo Receptor 1 (NgR1) containing receptor complex, while the Nogo-A-Δ20 receptor was unknown for a long time. Recently, we identified sphingosine 1-phosphate receptor 2 (S1PR2), a member of the G protein coupled receptor (GPCR) superfamily, as a receptor for the Nogo-A-Δ20 domain (Kempf et al., 2014). This is of particular interest because the function blocking anti-Nogo-A antibody 11C7, which enhances compensatory sprouting and functional recovery after CNS trauma, targets the Nogo-A-Δ20 domain.
While the immediate cascades of Nogo-A signalling leading to growth cone collapse and retraction are well established, little is known about the physiological role of Nogo-A in oligodendroglia. Nogo-A is expressed throughout the oligodendroglial lineage and the differentiation stage specific composition of the receptor complexes is currently under examination. We previously showed that systemic Nogo-A knock out mice exhibit a postnatal delay in myelination that normalizes after one month (Pernet et al., 2008). To investigate the cause of this transient hypomyelination, oligodendrocyte differentiation dynamics were analysed in postnatal Nogo-A knock out and wild type control mice by immunofluorescent stainings for stage specific markers. Preliminary results indicate that Nogo-A knock out animals show a delay in oligodendrocyte maturation. In contrast, no effect of Nogo-A on oligodendroglial survival was

found, indicating a specific role of Nogo-A in oligodendrocyte differentiation. Further research into the underlying mechanisms will be conducted to possibly find new ways of enhancing remyelination in myelin related diseases such as multiple sclerosis.

Disclosures: **M.A. Maibach:** None. **F. Müller:** None. **O. Weinmann:** None. **M.E. Schwab:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.16/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: US National Institutes of Health R01NS072427

US National Institutes of Health R01NS075243

National Multiple Sclerosis Society (NMSS-4727)

Title: MicroRNA control of myelination and remyelination in the CNS

Authors: ***H. WANG**, Z. MA, Y. DENG, R. LU;
Cincinnati Children's Hosp., Cincinnati, OH

Abstract: microRNAs (miRNAs) have been implicated in oligodendrogenesis and demyelinating diseases; however, underlying specific miRNAs have remained elusive. Through *in vivo* targeted mutagenesis in mice, we find that miR-219 is required for proper oligodendrocyte differentiation and myelination. Temporally-specific ablation reveals a critical role for miR-219 in oligodendrocyte remyelination after lysolecithin-induced demyelination, while miR-219 overexpression promotes precocious oligodendrocyte maturation and myelin regeneration. Accordingly, administration of miR-219 mimics to lysolecithin-induced demyelinating lesions in the murine spinal cord enhances myelin restoration. Through an integrated transcriptome profiling and biotin-affinity miRNA pull-down approach, we identify stage-specific targeting of differentiation inhibitors by miR-219, and further uncover novel sets of miR-219 targets including Etv5 and Lingo1 that inhibit oligodendrocyte maturation. Inhibiting *Etv5* and *Lingo1* leads to a partial rescue of differentiation defects in *miR-219*-deficient oligodendrocyte culture. Together, our findings identify context-specific miRNA-regulated checkpoints that control CNS myelinogenesis and myelin repair.

Disclosures: **H. Wang:** None. **Z. Ma:** None. **Y. Deng:** None. **R. Lu:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.17/B4

Topic: A.01. Neurogenesis and Gliogenesis

Support: SUNY Health Science Center Foundation

Title: Fyn-Dab1 signaling during oligodendrocyte differentiation

Authors: ***D. J. OSTERHOUT**, H. BHATTI, I. I. GENEVA;
Dept Cell & Developmental Biol, SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Oligodendroglial progenitor cells (OPCs) are the precursors to myelinating oligodendrocytes in the central nervous system (CNS). These cells are generated in the ventral neuroepithelium at later stages of cortical development, migrating into the cortex where they contact axons and ultimately form a myelin membrane. During the process of differentiation, OPCs undergo significant morphological changes, extending many branched processes which will make contact with axons. Once in contact with an axon, the oligodendrocyte process expands and begins to form the myelin membrane which will ensheathe the axon.
The morphological differentiation of oligodendroglial progenitor cells, from a simple bipolar cell to a cell with multiple complex processes, is a key requirement for myelin formation. The activation of the tyrosine kinase Fyn is an early step in the differentiation of oligodendroglial progenitor cells. Fyn activation occurs in oligodendroglial progenitor cells even before any changes in cellular morphology are observed. Once active, Fyn regulates the morphological differentiation of these cells, initiating process outgrowth and myelin sheet formation *in vitro.* In Fyn deficient mice, myelin formation is markedly reduced, demonstrating the importance of this kinase in myelination *in vivo*.
Fyn interacts with many downstream effectors, including molecular signaling pathways that interact with the cytoskeleton and initiate process outgrowth.This includes the adaptor protein Dab1. Our early work has demonstrated that Fyn-Dab1 interactions are important for OPC migration, as animals deficient in either Fyn or Dab1 show reduced OPC migration from the subventricular zone *in vivo*. In the present study, we show that Fyn-Dab1 interactions with cytoskeletal components in the cell are important for the initiation of process outgrowth.

Disclosures: **D.J. Osterhout:** None. **H. Bhatti:** None. **I.I. Geneva:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.18/B5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIAAA P50-AA022534

R37-AA015614

Title: Acute oligodendrocyte loss and persisting white matter injury in a third trimester equivalent model of fetal alcohol spectrum disorder

Authors: J. C. NEWVILLE[1], C. F. VALENZUELA[1], L. LI[1], L. L. JANTZIE[2], *L. A. CUNNINGHAM[1];
[1]Neurosciences, [2]Pediatrics, Univ. of New Mexico Sch. of Med., Albuquerque, NM

Abstract: Recent human imaging studies have linked white matter abnormalities in the corpus callosum with cognitive impairment in children with fetal alcohol spectrum disorder (FASD), however, the underlying mechanisms remain unknown. The Nestin-CreER^{T2}/tdTomato strain was used in the current study to evaluate the acute and persisting impacts of alcohol exposure on oligodendrocyte number and white matter integrity in a third-trimester equivalent mouse model of FASD. Nestin-CreER^{T2}/tdTomato pups received a single intraperitoneal injection of tamoxifen (33 mg/kg) at postnatal day 2 (P2) to label oligodendrocyte lineage cells derived from the postnatal subventricular zone (SVZ; Olig2^{+}/tdTomato^{+}), distinguishing them from those that originated during embryonic development (Olig2^{+}/tdTomato^{-}). Cages containing both mothers and pups were placed into inhalation chambers where they were exposed to ethanol vapor or air for four hours daily from P3 through P15, resulting in a mean pup blood ethanol concentration of 160.4 ± 12.0 mg/dl (range = 128.2 – 185.6 mg/dl). Stereological analysis in septal corpus callosum of tdTomato^{-} oligodendrocytes (OLs) demonstrated a 53% decrease in the number of mature OLs (CC1^{+}/Olig2^{+}; EtOH 20,162 ± 3,944 cells/mm^3, n=3; Air 42,804 ± 5,058 cells/mm^3, n=5; p=0.0125), and a 75% decrease in proliferating oligodendrocyte progenitor cells (OPCs; Ki67^{+}/Olig2^{+}; EtOH 1,608 ± 658.0 cells/mm^3, n=3; Air 6,276 ± 1,232 cells/mm^3, n=5; p=0.0169) at P16 following early postnatal ethanol exposure. By P50 both tdTomato^{-} mature OL and proliferating OPC numbers recovered compared to those of air controls. Interestingly, there was no effect of alcohol on the numbers of tdTomato^{+} OLs or OPCs cells at either P16 or P50, suggesting heterogeneity of oligodendrocyte alcohol susceptibility dependent on ontogenetic origin. Further characterization of white matter injury in the corpus callosum at P50 indicated persisting myelin protein dysregulation and microstructural abnormalities of alcohol-exposed subjects, as assessed by western immunoblotting of myelin basic protein (MBP; increased

expression) and MRI diffusion tensor imaging (DTI; decreased fractional anisotropy). These results demonstrate that third-trimester equivalent alcohol exposure leads to an acute, transient decrease in OL lineage cell numbers, accompanied by enduring white matter injury. Additionally, our finding of heterogeneity in alcohol susceptibility based on the developmental origin of OLs may have therapeutic implications in FASD and other disorders of white matter development.

Disclosures: **J.C. Newville:** None. **C.F. Valenzuela:** None. **L. Li:** None. **L.L. Jantzie:** None. **L.A. Cunningham:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.19/B6

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01 NS073425

NIH Grant R01 NS074870

NMSS Grant RG4579

Title: Heterogeneity of astrocyte and NG2 cell insertion at the node of Ranvier

Authors: ***P. JUKKOLA**, D. R. SERWANSKI, A. NISHIYAMA;
Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: The node of Ranvier is a functionally important site on the myelinated axon where sodium channels are clustered at a high density, allowing rapid saltatory conduction of action potentials. Early ultrastructural studies have revealed the presence of “glia” or “astrocytes” at the nodes. NG2 cells, also known as oligodendrocyte precursor cells or polydendrocytes, comprise a population of resident glial cells in the mature mammalian central nervous system that is distinct from astrocytes, and processes of NG2 cells have been detected at the nodes. However, the frequency of the occurrence of the two types of glia at the node has remained unknown. We have used specific cell surface markers to examine the prevalence of NG2 cells and astrocytes at the node of Ranvier in the optic nerve, corpus callosum, and spinal cord of young adult mice or rats. We show that >96% of the nodes in all three areas contained astrocyte processes, while 33-49% of nodes contained processes from NG2 cells. Only a few nodes appeared devoid of glial apposition. Intriguingly, NG2 cell processes showed a clear preference for larger nodes of

Ranvier. The heterogeneity of glial processes at the nodes was confirmed by stimulated emission depletion (STED) confocal microscopy and by electron microscopy. Electron microscopy using unlabeled and pre-embedding immunolabeled tissue revealed differences in the spatial relationship of each type of glia to the nodal structure. These findings provide a morphological basis for future investigations on the role of NG2 cells and astrocytes in nodal function and white matter plasticity.

Disclosures: P. Jukkola: None. **D.R. Serwanski:** None. **A. Nishiyama:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.20/B7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NINDS R01NS083947-01

Title: Glycogen synthase kinase 3 β inhibition promotes myelination in preterm rabbit pups with intraventricular hemorrhage

Authors: *P. DOHARE, F. HU, P. BALLABH;
Pediatrics, Cell biology and Anat., New York Med. Col., Valhalla, NY

Abstract: Intraventricular hemorrhage (IVH) in preterm infants results in white matter injury and neurological deficits. Reduced myelination in infants with IVH is attributed to reduced proliferation and arrested maturation of oligodendrocyte progenitor cells (OPCs). Glycogen synthase kinase 3 β (Gsk-3β) proteins regulate proliferation and maturation of neural cells during development. Studies have shown that Gsk-3β inhibition enhances proliferation and differentiation of OPCs and myelination of the white matter in neonatal rats as well as in adult models of demyelination. Therefore, we hypothesized that Gsk-3β inhibition might promote multiplication and maturation of OPCs, and accelerate myelination in rabbit pups with IVH. To test our hypotheses, we employed preterm rabbit (E29, Term=32d) model of glycerol-induced IVH. We treated pups with IVH by ARA-014418 (ARA, 20 mg/kg twice a day), a Gsk-3β inhibitor, or vehicle for 7 days, and compared the expression of myelin basic protein (MBP), myelin associated glycoprotein (MAG), and GFAP between ARA- and vehicle-treated pups with IVH at postnatal day (D) 14 using immunohistochemistry and Western blot analyses. We found that ARA treatment increased phospho-Gsk-3β levels (serine9), however did not affect activate β-catenin or TCF4 levels at D3 in preterm pups with IVH, suggesting failure of ARA to

upregulate Wnt signaling in pups with IVH despite evidence of its activity. More importantly, we noted that MBP and MAG levels were elevated in ARA treated pups with IVH compared to vehicle controls at D 14 on both immunohistochemistry and Western blot analyses ($P<0.05$, all comparisons). ARA treatment did not affect GFAP expression in pups with IVH. NICD (Notch intracellular domain) levels were significantly reduced in ARA treated pups relative to controls ($P<0.05$) and Hes5 levels showed a trend toward decrease in ARA treated pups. Additionally, ARA treatment increased Sox2 and reduced Dlx1 levels in pups with IVH. Data suggest that Gsk-3β inhibition by ARA treatment promotes myelination in rabbit pups with IVH. Notch inhibition, an increase in neural progenitor pool, and a shift in the fate of multipotent progenitors from interneuron precursors to OPCs might be contributing to enhanced myelination in rabbits with IVH. We speculate that ARA treatment might promote myelination and neurological recovery in human premature infants with IVH.

Disclosures: **P. Dohare:** None. **F. Hu:** None. **P. Ballabh:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.21/B8

Topic: B.12. Glial Mechanisms

Support: JSPS KAKENHI (25430079)

JSPS KAKENHI (26117519)

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JSPS KAKENHI (15K18381)

JSPS KAKENHI (16K07073)

Sakamoto Research Foundation of Psychiatric Diseases

Title: Association between stress-induced Ranvier nodes structural abnormalities and reduced oligodendrocyte activity in major depressive disorder

Authors: *S. MIYATA, S. SHIMIZU, T. TANAKA, M. TOHYAMA;
Kindai Univ/ Res. Ins Trad Asian Med., Osaka-Sayama, Osaka, Japan

Abstract: Major depressive disorder is probably the oldest and still one of the most frequently diagnosed psychiatric illnesses. Major depressive disorder is one of the leading causes of disturbances in emotional, cognitive, autonomic, and endocrine functions, affecting nearly 7% of the population in Japan. According to the large amount of information on depressive diseases that has been accumulated during recent years, patients with major depressive disorder show an enhanced biologic stress-response mechanism, especially a hyperactive hypothalamic-pituitary-adrenal (HPA) axis and high levels of circulating cortisol. Although dysregulation of the HPA axis by chronic stress is indicative of major depressive disorder, the molecular mechanisms and functional changes in the brain underlying depression are largely unknown. In this study, we have developed an animal model of depression by exposing mice to chronic stress. These mice show depression-like symptoms including chronically elevated plasma levels of corticosterone. We previously showed oligodendrocyte (OL)-specific activation of the serum/glucocorticoid-regulated kinase (SGK)1 cascade, increased expression of axon-myelin adhesion molecules, and elaboration of the oligodendrocytic arbor in the corpus callosum of chronically stressed mice. In the current study, we demonstrate that the nodes and paranodes of Ranvier in the corpus callosum were narrower in these mice. Chronic stress also led to diffuse redistribution of Caspr and Kv 1.1 and decreased the activity in white matter, suggesting a link between morphological changes in OLs and inhibition of axonal activity. OL primary cultures subjected to chronic stress resulted in SGK1 activation and translocation to the nucleus, where it inhibited the transcription of metabotropic glutamate receptors (mGluRs). Furthermore, the cAMP level and membrane potential of OLs were reduced by chronic stress exposure. We showed by diffusion tensor imaging that the corpus callosum of patients with MDD exhibited reduced fractional anisotropy, reflecting compromised white matter integrity possibly caused by axonal damage. Our findings suggest that chronic stress disrupts the organization of the nodes of Ranvier by suppressing mGluR activation in OLs, and that specific white matter abnormalities are closely associated with MDD onset.

Disclosures: **S. Miyata:** None. **S. Shimizu:** None. **T. Tanaka:** None. **M. Tohyama:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.22/B9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS052741

NMSS RG4958

Mayo Clinic Center for Regenerative Medicine

Title: Protease activated receptor 2 is a novel regulator of myelin development and repair.

Authors: *I. A. SCARISBRICK[1], M. RADULOVIC[2], H. YOON[2];
[2]Physical Med. and Rehabil., [1]Mayo Clin., Rochester, MN

Abstract: Oligodendrocytes are essential regulators of nerve impulse conduction and energy homeostasis in the developing and adult central nervous system and therefore represent an important target for the design of therapies to promote recovery of function in cases of injury and disease. In this study, we evaluated the role of a unique protease activated G-protein coupled receptor referred to as protease activated receptor 2 (PAR2) in spinal cord myelin development and repair. PAR2 is a seven transmembrane G-protein-coupled receptor that becomes activated upon enzymatic cleavage within its extracellular domain. PAR2 is therefore positioned to serve as a key translator of the proteolytic microenvironment into cellular responses that regulate myelin homeostasis and regeneration. A systematic comparison of patterns of myelination in the spinal cord of wild type and PAR2 knockout mice revealed that those with PAR2 loss-of-function exhibited earlier onset of myelin production and overall acceleration of myelination during the postnatal period and into adulthood. The pro-myelinating effects of PAR2-loss-of-function included higher proteolipid protein (PLP) levels at birth, more Olig2+ oligodendrocytes at P7, and more mature CC1+ myelinating cells by P7 and P21. Moreover, the spinal cord of mice lacking PAR2 displayed overall higher levels of myelin basic protein (MBP) and thickened myelin sheaths in adulthood compared to mice with an intact PAR2 signaling system. Enhancements in myelination with PAR2 loss-of-function were accompanied by increases in the pro-myelination signaling pathway pERK1/2 within the spinal cord from P7 onwards. Supporting a direct role for PAR2 as a suppressor of myelination, oligodendrocyte progenitor cells (OPCs) derived from PAR2-null mice, or wild type OPCs treated with a PAR2 small molecule inhibitor, expressed higher levels of the major myelin proteins PLP and MBP. PAR2 loss-of-function also improved the preservation of myelin in the context of traumatic spinal cord injury. Collectively, these studies point to signaling at PAR2 as a novel suppressor spinal cord myelination during postnatal development and as a critical regulator of myelin integrity after injury of the adult spinal cord. Therefore, PAR2 represents a new target for therapies aimed at promoting myelination developmentally and myelin preservation in neurological disorders such as spinal cord injury where white matter injury is a central concern.

Disclosures: I.A. Scarisbrick: None. **M. Radulovic:** None. **H. Yoon:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.23/B10

Topic: A.01. Neurogenesis and Gliogenesis

Title: Motor neuron sonic hedgehog regulates oligodendrocyte proliferation and maturation in the developing spinal cord

Authors: *L. STARIKOV[1,2], A. KOTTMANN[1,2];
[1]Physiology, Pharmacol. and Neurosci., Sophie Davis Sch. of Biomed. Education, CUNY, New York, NY; [2]Molecular, Cellular, and Developmental Biol., CUNY Grad. Ctr., New York, NY

Abstract: Sonic hedgehog (Shh) secreted from transient organizing tissues such as notochord and floorplate during development determines neuronal and glial cell fate and exemplifies the classic principle of a morphogen. We find that besides the floorplate, post mitotic motor neurons (MNs) are an additional ventral source of Shh important for regulating the specification and maturation of oligodendrocyte precursors (OPCs) originating from the Olig2 domain during spinal cord development. Consistent with the anterior to posterior birth wave of oligodendrocytes in the spinal cord, we find a delay in the generation and migration of OPCs in Olig2cre; $Shh^{L/L}$ mutant animals that have Shh ablated from selectively MNs in an anterior to posterior pattern. Although OPC numbers and density seem to recover by E16.5 in mutant animals, OPC morphology in mutants is affected into postnatal life, leaving open the possibility of motor neuron Shh dependent lasting epigenetic regulation of OPCs during a critical window of OPC generation from the Olig2 domain.

Disclosures: L. Starikov: None. **A. Kottmann:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.24/B11

Topic: A.01. Neurogenesis and Gliogenesis

Support: Swiss National Science Foundation

Advanced ERC grant (Nogorise) to M.E. Schwab

Title: Nogo/RTN4 as an extracellular vesicle-associated ligand

Authors: *M. M. HOLM[1], D. VAN ROSSUM[1], M. EGGER[1], O. WEINMANN[1], I. K. HERMANN[2], M. E. SCHWAB[1];
[1]Brain Res. Inst., Zuerich, Switzerland; [2]EMPA Swiss Federal Labs. for Materials Sci. and Technol., St. Gallen, Switzerland

Abstract: The reticulons (RTNs) are a family of transmembrane proteins found in the endoplasmic reticulum (ER) as well as in smaller though functionally relevant quantities at the plasma membrane. They all share a common C-terminal transmembrane reticulon homology domain (RHD); however, the N-termini of RTNs differ greatly with respect to length, structure and function. The most studied member of this family, Nogo-A (RTN4A), has an almost 1000 amino acid long N-terminus important for the signaling function of the protein as an inhibitor of neurite outgrowth and a regulator of synaptic plasticity in the central nervous system (CNS). Nogo-B (RTN4B), a splice variant of Nogo-A, has a much shorter N-terminus of approximately 170 amino acids which it shares with the very N-terminus of Nogo-A. The function of Nogo-B is nonetheless much less clear though it has been described as a regulator of apoptosis and vascular remodeling.
The current view of Nogo signaling is that plasma membrane-bound full-length Nogo initiates signaling cascades through binding to receptors on cells in direct contact with the Nogo expressing cell. However, the presence of Nogo sequences in bodily fluids such as the cerebrospinal fluid (CSF) has been anecdotally reported and recently found in a proteomic study (Chiasserini *et al.*, 2014). As the presence of functionally active Nogo in the CSF and other bodily fluids would have major implications e.g. for the *in vivo* administration of anti-Nogo-A antibodies as therapies, we sought to investigate whether either full-length or cleaved fragments of Nogo are released into the supernatant of cultured neuron-like cells. We found that full-length Nogo-A, full-length Nogo-B, as well as cleaved fragments which include the shared N-terminus of Nogo-A/-B, are found in the culture supernatant of N2a neuroblastoma cells. Both the full-length proteins as well as the cleaved fragments were associated with extracellular vesicles rather than free in solution. Furthermore, we found that extracellular vesicles from the cerebrospinal fluid (CSF) of adult rats are enriched in full-length Nogo-A, but not Nogo-B or the N-terminal cleaved fragments. Current work is focused on assessing the functionality of vesicle-bound Nogo-A as a ligand, and evaluating the significance of the proteolytic processing of Nogo-A/B in the context of Nogo signaling.

Disclosures: **M.M. Holm:** None. **D. van Rossum:** None. **M. Egger:** None. **O. Weinmann:** None. **I.K. Hermann:** None. **M.E. Schwab:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.25/B12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01-NS056427

NMSS RG 4706A4/2

Title: Sox17 suppression of Wnt/beta-catenin promotes oligodendrocyte regeneration through Hedgehog-Smoothened signaling

Authors: *L.-J. CHEW, X. MING, B. MCELLIN, V. GALLO;
Ctr. Neurosci Res., Children's Res. Inst., Washington, DC

Abstract: Sox17 is the only Sox F transcription factor to be analyzed in the brain, and it has been shown to promote oligodendrocyte (OL) development. Sox17 is expressed in regenerating OLs of adult white matter (WM) lesions, but its role in repair is not understood. Sox17 overexpression in CNPSox17 transgenic mice was found to promote postnatal OL development and prevent OL loss after focal lysolecithin (Lyso) demyelination. Our present studies show that CNPCre-mediated Sox17 ablation (cko) results in a transient developmental decrease in postnatal Olig2+ OL lineage cells at P30, which is followed by recovery. WM recovery after Lyso demyelination is delayed in the cko as lesions fail to regain MBP expression at 14DPL, accompanied by reduced nascent MAG+ cells. Since Sox17 inhibits Wnt/beta-catenin signaling in vitro, its ablation in vivo may elevate beta-catenin activity. Increased unphosphorylated beta-catenin (active, ABC) levels were found in the cko, which were further increased after Lyso. Conversely, demyelination-induced ABC was not observed in CNPSox17 lesions. Fewer ABC+Iba1+ microglia and ABC+Caspase3+ cells indicated attenuated damage and reactivity. OL regeneration was increased, evidenced by increased BrdU+Olig2+ cells. Reduced b-catenin activation may promote progenitor maturation to OLs, which were increased in CNPSox17 lesions. Stereotaxic injection of the b-catenin antagonist CCT036477 into C57Bl6 mouse lesions not only improved OL generation, but also prevented Caspase3+ cell increase. Although total beta-catenin was increased in intact CNPSox17 WM, ABC levels did not increase proportionately, suggesting tonic repression by Sox17. Surprisingly, Sox17 was not detected in b-catenin immunoprecipitates from adult CNPSox17 WM, so that b-catenin inhibition may be indirect. Since GLI2 was previously found to be elevated in CNPSox17, Hedgehog signaling is a possible candidate. PDGFR-Cre-mediated ablation of *Gli2* or Smoothened (*Smo*) at P60 increased ABC+ cells in intact WM, indicating Hedgehog repression of Wnt/b-catenin. Pharmacological GLI inhibition renders the CNPSox17 WM vulnerable to Lyso damage.

Stereotaxic application of the SMO agonist SAG in C57Bl6 Lyso lesions, similarly to CNPSox17, prevented the ABC increase and promoted OPC differentiation, supporting Hedgehog-mediated OL regeneration. This is consistent with enhanced Smo activation in CNPSox17 lesions, and *Smo* ablation in CNPSox17 lesions results in a large increase in ABC at 3DPL. These studies in Sox17 mutant strains thus reveal a negative regulatory relationship between Hedgehog and Wnt/b-catenin that is integrated and enhanced by Sox17 during OL regeneration.

Disclosures: **L. Chew:** None. **X. Ming:** None. **B. McEllin:** None. **V. Gallo:** None.

Poster

390. Oligodendrocytes and Schwann Cells: Development, Neuron-Interaction, and Myelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 390.26/B13

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01-NS079166

NIH Grant R01-DA036165

Title: Pain-induced spinal NG2 cell proliferation: a critical role of β-catenin in neurons but not in NG2 cells

Authors: *Y. SHI, S.-J. TANG;
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Abstract: NG2 neural stem cells are oligodendrocyte precursors that critically contribute to myelination during development and/or remyelination in response to central nervous system (CNS) injuries. Emerging evidence suggests that neuronal activity intimately regulates the proliferation of NG2 cells, probably via the neuron-NG2 synapses. We hypothesize that NG2 cells in the pain neural pathway respond to nociceptive stimulation and potentially regulate pain pathogenesis. To test this hypothesis, we injected capsaicin (i.d.) in the mouse hind paw and quantified NG2+ cells in the spinal dorsal horn (SDH). We observed a significant increase of NG2+ cells in the superficial layers of the SDH in the capsaicin-treated group. About 70% of NG2+ cells were also Olig2-positive, indicating that they were progenitors of oligodendrocytes. Bromodeoxyuridine (BrdU) labeling experiments indicated that capsaicin-induced NG2+ cell increase was resulted from cell proliferation. To understand the mechanism by which pain signals stimulate NG2 cell proliferation, we tested the role of β-catenin, because recent studies

suggest its important role in regulating NG2 cell proliferation and differentiation during development or remyelination. Intrathecal injection (i.t.) of β-catenin inhibitor XAV939 blocked capsaicin-induced NG2+ cell increase. To specifically determine the role of β-catenin, we generated conditional knockout (CKO) mice. Surprisingly, deletion of β-catenin in NG2 cells did not attenuate capsaicin-induced NG2+ cell increase. In contrast, deletion of one copy of β-catenin in neurons blocked the increase of NG2+ cells. These unexpected findings suggest that β-catenin in neuron might be critical for generating a neuron-to-NG2 signal that elicits NG2 cell proliferation in response to painful stimulation.

Disclosures: Y. Shi: None. **S. Tang:** None.

Poster

391. Autism: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 391.01/B14

Topic: A.07. Developmental Disorders

Support: Korea Healthcare Technology R&D Project (HI3C1451)

Science and Technology (NRF-2011-0021866).

Title: Early behavioral abnormalities and perinatal alterations of PTEN/AKT pathway in valproic acid atuism model mice

Authors: *E. YANG[1], S. AHN[2], K. LEE[1], U. MAHMOOD[1], H.-S. KIM[3];
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Abstract: Exposure to valproic acid (VPA) during pregnancy has been linked with increased incidence of autism, and has repeatedly been demonstrated as a useful autism mouse model. We examined the early behavioral and anatomical changes as well as molecular changes in mice prenatally exposed to VPA (VPA mice). In this study, we first showed that VPA mice showed developmental delays as assessed with self-righting, eye opening tests and impaired social recognition. In addition, we provide the first evidence that primary cultured neurons from VPA-treated embryos present an increase in dendritic spines, compared with those from control mice. Mutations in phosphatase and tensin homolog (PTEN) gene are also known to be associated with autism, and mice with PTEN knockout show autistic characteristics. Protein expression of PTEN was decreased and the ratio of p-AKT/AKT was increased in the cerebral cortex and the

hippocampus, and a distinctive anatomical change in the CA1 region of the hippocampus was observed. Taken together, our study suggests that prenatal exposure to VPA induces developmental delays and neuroanatomical changes via the reduction of PTEN level and these changes were detectable in the early days of life.

Disclosures: **E. Yang:** A. Employment/Salary (full or part-time): BK 21 plus. **S. Ahn:** None. **K. Lee:** None. **U. Mahmood:** None. **H. Kim:** Other; he Korea Healthcare Technolog y R&D Project (HI3C1451) of Ministry for Health,, Science and Technology (NRF-2011-0021866).

Poster

391. Autism: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 391.02/B15

Topic: A.07. Developmental Disorders

Title: MicroRNAs as potential biomarkers in Autism spectrum disorder

Authors: *M. NAKATA[1], R. KIMURA[1], K. TOMIWA[3], T. AWAYA[2], T. KATO[2], Y. FUNABIKI[4], T. HEIKE[2], M. HAGIWARA[1];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and a pattern of repetitive stereotyped behaviors. Many studies have been reported that high rates of mental health problems are observed commonly in ASD, especially depression and anxiety. Therefore, early detection is important to intervene early and prevent later psychiatric symptoms. Despite all the research efforts, however, reliable diagnostic biomarkers are not established as it stands. MicroRNAs (miRNAs) are a class of short non-coding RNAs that regulate gene expression. Increasing evidence has shown that miRNAs may play essential roles in neurodevelopmental disorders.
In this study, we evaluated blood-based microRNAs as novel biomarkers for diagnosis of ASD. We determined miRNA expression profiles of ASD using Agilent miRNA microarray analysis in discovery sample set (30 ASD patients and 30 Controls). An independent replication sample set was used for the biomarker validation by real-time RT-PCR. Further we constructed ROC (Receiver Operating Characteristics) curves to determine the sensitivity and specificity of individual miRNA as a diagnostic biomarker. We found that three miRNAs had significantly altered in ASD patients compared to healthy controls and also got a good ROC curve according

to the accepted classification of biomarker utility. These miRNAs were expressed in not only blood but also human brain. Our findings provide novel and reliable biomarkers for diagnosis of ASD.

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Poster

391. Autism: Models

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: CAS Strategic Priority Research Program XDB02050400

NSFC Grants #91432111

Title: Autism-related protein MeCP2 regulates FGF13 expression and emotional behaviours

Authors: *B. YUAN;
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Abstract: Background
MeCP2 protein functions through either repressing gene expression transcriptionally by recruiting HDAC complexes or post-transcriptionally suppressing nuclear microRNA processing. Mutations and copy number variations of the MECP2 gene are associated with Rett syndrome and autism spectrum disorders. FGF13 is a microtubule binding protein and plays a critical role in regulating neural development. Loss of the FGF13 gene leads to severe intellectual disabilities in human.
Results
Here we found that MeCP2 regulates the expression of FGF13 via two distinct microRNAs. The protein level of FGF13 decreased in Mecp2 knockout mice and elevated in MeCP2 overexpression mice, indicating the regulatory cascade of MeCP2 to FGF13. Importantly, we found that abnormal elevated fear conditioning behaviors in MeCP2 overexpression mice overcome the defective fear learning behaviors of FGF13 knockout mice, suggesting the molecular axis of regulating fear learning and memory by the MeCP2-FGF13 regulatory cascade. However, the anxiety behaviors of MeCP2 overexpression and FGF13 knockout mice seem not to compensate with each other.
Conclusions

This work provides a functional connection between two critical disorder-related genes, MeCP2 and FGF13, further suggesting that behavioral abnormalities found in mouse models of brain disorders may be compensated with each other, if genetic causes are within the same regulatory axis.

Disclosures: B. Yuan: None.

Poster

391. Autism: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 391.04/B17

Topic: A.07. Developmental Disorders

Title: Shank2 and Shank3 gene - environment interactions in autism spectrum disorders

Authors: *S. GRABRUCKER[1], G. EHRET[2], T. M. BOECKERS[3], A. M. GRABRUCKER[4]; [2]Neurobio., [3]Anat. and Cell Biol., [4]Neurol., [1]Ulm Univ., Ulm, Germany

Abstract: Autism Spectrum disorders (ASD) are neurodevelopmental disorders characterized by impairments in communication and social behavior, and by repetitive behaviors. ASD is considered to be a multi-factorial disorder resulting from genetic and non-genetic risk factors and their interaction. As genetic factors of ASD, mutations and deletions in SHANK genes have been identified that interfere with synaptogenesis and synapse function. However, ASD are also influenced by a variety of environmental, and possibly immunological factors that act during critical periods of brain development. The interaction of environmental exposures in the context of an individual's genetic susceptibility may manifest differently in each case and thus may be responsible for the heterogeneous phenotypes and varied comorbidities within the disorder. Here, using mouse models, we show that maternal zinc status as risk factor for ASD intersects with the SHANK pathway and the deregulation of any of these two factors may modify the behavior of the offspring leading to ASD. In particular, our data show that low postsynaptic zinc availability impacts the activity dependent regulation of Shank2 and Shank3 at the synapse and that a loss of synaptic Shank2 and Shank3 occurs in a mouse models for prenatal zinc deficiency. Zinc is one of the most prevalent metal ions in the brain and participates in processes such as neurogenesis, neuronal migration and differentiation, thereby shaping brain development and function. Similar to SHANK KO mice, prenatal zinc deficient animals displayed ASD related behavior later in life such as deficits in vocalization, increased aggression and slight abnormalities towards social stimuli and in social situations. Taken together, we suggest that part of the molecular underpinning of prenatal zinc deficiency as a risk factor for ASD may unfold through the

deregulation of zinc-binding Shank family members in the brain but also the gastro-intestinal system.

Disclosures: S. Grabrucker: None. **G. Ehret:** None. **T.M. Boeckers:** None. **A.M. Grabrucker:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.05/B18

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 26700012

JSPS KAKENHI 23300151

Title: A rubber tail task in Ca^{2+}-dependent activator protein for secretion (CAPS) 2 knockout mice

Authors: *M. WADA[1,2], M. IDE[1,3], T. ATSUMI[1], K. YAGISHITA[4], M. KATAKAI[4], Y. SHINODA[4,5], T. FURUICHI[4], K. KANSAKU[2,6];
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Abstract: In human, when a rubber hand and a participant's hand are synchronously stroked by two brushes, a participant feels as if the rubber hand becomes his/her own hand (rubber hand illusion, RHI). We delivered the synchronous stroking to a rubber tail and a real tail of the mouse, and found that wild type mice responded (e.g., orienting or retracting the head) as if their own tails were being touched when the rubber tails were grasped (a RHI-like phenomenon in mice). From this discovery, we have developed a rubber tail task in mice, aiming to evaluate their body ownership. The RHI in human is known to be weak in persons with autism spectrum disorder (ASD). Thus, we examined whether the RHI-like phenomenon occurred in Ca^{2+}-dependent activator protein for secretion 2 knockout (CAPS2 KO) mice. The CAPS2 gene is known to involve in the release of brain-derived neurotrophic factor (BDNF), and its knockout (CAPS2 KO) mice show autistic-like phenotypes.
The CAPS2 KO mice (n = 5) and wild type mice (n = 13) were trained to stay in a small tube,

and were received daily tests under two conditions with two brushes: 1) synchronous stroking of a real tail and a rubber tail, 2) asynchronous stroking of both tails. After the tails were stroked for more than 1 minute, an experimenter firmly grasped the rubber tail, and responses of the mice were recorded and analyzed. A mean response rate over 10 days of testing in each condition was calculated.
Following the stroking, the response rates were significantly larger in the synchronous stroking condition (mean ± standard error, 0.41 ± 0.025), compared to the asynchronous stroking condition (0.26 ± 0.024), as previously reported in wild type mice. In contrast, there was no difference in the response rates between the synchronous (0.31 ± 0.033) and asynchronous (0.32 ± 0.035) stroking conditions in CAPS2 KO mice. Differences in response rates between conditions were significantly larger in the WT mice ($P < 0.001$, Wilcoxon rank sum test).
Our result suggests that the RHI-like phenomenon might not occur in CAPS2 KO mice. Dysfunctions in body ownership in ASD were partly simulated in the mouse model of ASD. Disorders in development of GABAergic interneuron network in CAPS2 KO mice may involve deficits in body ownership illusions in addition to the deficits in social communications.

Disclosures: **M. Wada:** None. **M. Ide:** None. **T. Atsumi:** None. **K. Yagishita:** None. **M. Katakai:** None. **Y. Shinoda:** None. **T. Furuichi:** None. **K. Kansaku:** None.

Poster

391. Autism: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 391.06/B19

Topic: A.07. Developmental Disorders

Title: The effects of vagus nerve stimulation on abnormal emotional learning and social anxiety in an animal model of autism

Authors: *A. ALVAREZ-DIEPPA, S. CAVALIER, K. GRIFFIN, C. MCINTYRE;
Univ. of Texas At Dallas, Richardson, TX

Abstract: Administration of vagus nerve stimulation (VNS) during trials of fear extinction (VNS-paired extinction) enhances extinction of fear in healthy rats. Our studies show that VNS-paired extinction affects plasticity related proteins like CaMKII, Arc, and the GluN2B subunit of NMDA receptors in the basolateral complex of the amygdala (BLA; an area of the brain important for fear learning and social behaviors). Rats prenatally exposed to 500 mg/kg valproic acid (VPA-exposed rat) are used as an animal model of autism and they show impaired extinction of fear and diminished social interaction. We studied the potential of VNS to reverse fear extinction and social interaction impairments in rats that were prenatally exposed to VPA.

Male VPA-exposed and saline-control rats were subjected to auditory fear conditioning followed by extinction training that was paired with VNS or sham stimulation. Another cohort was exposed to a social interaction task during which entries to the social zone were coupled with VNS or sham stimulation. All groups showed the same level of conditioned fear after training. VPA-exposed rats given VNS during extinction training showed freezing levels that were similar to those of saline-control rats, and significantly lower than those of VPA-exposed rats given sham stimulation. VPA-exposed rats given VNS while in the social zone demonstrated higher preference for the social zone compared to VPA-exposed rats given sham stimulation, and showed a sociability index similar to that of saline-control rats. Western blot analysis revealed a significant decrease in phosphorylated CaMKII, Arc protein, and GluN2B expression in the BLA of VPA-exposed rats compared to saline-control rats. Results suggest that VNS-paired extinction can rescue extinction learning, and VNS-paired social interaction can alleviate social interaction deficits in VPA-exposed rats. Furthermore, abnormal CaMKII, Arc, and GluN2B expression in the BLA may underlie some of the cognitive deficits seen in the VPA-exposed rat model of autism.

Disclosures: **A. Alvarez-Dieppa:** None. **S. Cavalier:** None. **K. Griffin:** None. **C. McIntyre:** None.

Poster

391. Autism: Models

Location: Halls B-H

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Program#/Poster#: 391.07/B20

Topic: A.07. Developmental Disorders

Support: This study was supported by a funding by the European Autism Interventions—A Multicentre Study for Developing New Medications (EU-AIMS) to Dr. Martien J. Kas

Title: The behavioral expression and genetic regulation of repetitive and restricted behaviors in mice in the context of autism spectrum disorder

Authors: *R. T. MOLENHUIS[1], H. BRUINING[2], M. J. V. BRANDT[1], P. E. VAN SOLDT[1], J. P. H. BURBACH[1], F. A. IRAQI[3], R. MOTT[4], M. J. H. KAS[1];
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Abstract: Repetitive and restricted patterns of behavior are one of the predominant features of Autism Spectrum Disorder (ASD). They reflect reduced behavioral flexibility and inability to

adapt to novel situations. This behavioral domain is underrepresented in animal model studies of ASD. Here, we present novel assays of repetitive and restricted patterns of behavior in a mouse model of ASD and we test their genetic regulation using quantitative genetic mapping.
Repetitiveness in spontaneous exploratory behavior and locomotor-patterns was assessed in the BTBR T+tf/J mouse, an established behavioral model of ASD. A single-trial exposure to four novel objects showed that BTBR T+tf/J mice display a three-fold increase in the time spent in repetitive locomotor-patterns compared to C57BL/6J control mice as quantified by temporal pattern analysis of spatial bins of locomotor-activity. Significance was retained after correction for individual differences in locomotor activity ($p<0.001$).
We studied the genetic regulation of these behaviors using a novel mouse model population of human genetic diversity, the Collaborative Cross (CC). The quantitative expression of the novel object locomotor-patterns was highly variable and heritable. Quantitative trait locus (QTL) mapping in CC mice led to the identification of multiple genetic loci for different types of repetitive behaviors (genome-wide permuted $p<0.01$). Genetic loci for repetitive behaviors were partially linked to locomotor activity, suggesting that activity-levels and repetitive behaviors can have common but also differential genetic origins.
We show that unbiased analysis of locomotor patterns during novelty exposure can be used for quantitative analysis of restricted and repetitive behaviors. Using a combination of an established behavioral model of ASD and forward genetic analysis, we show that these phenotypes may be useful to dissect ASD's complex behavioral biology.

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Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant HD055751

Title: Behavioral effects of an m_1 muscarinic cholinergic receptor agonist in the BTBR mouse model of autism

Authors: *M. E. RAGOZZINO[1], H. RAMIREZ[2], J. T. DUNN[2], W. S. MESSER, Jr.[3]; [2]Psychology, [1]Univ. of Illinois at Chicago, Chicago, IL; [3]Pharmacol. and Exptl. Therapeut., Univ. of Toledo, Toledo, OH

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social communication and interaction deficiencies along with restricted interests and repetitive behaviors (RRBs). RRBs can include an insistence on sameness or behavioral inflexibility, as well as repetitive, stereotyped behaviors. At present, there are no approved treatments to alleviate RRBs in ASD. Results from post-mortem studies suggest that brain muscarinic cholinergic receptor activity may be reduced in ASD. Thus, treatment with a muscarinic cholinergic receptor agonist may be effective in alleviating core symptoms in ASD. A recent study with BTBR mice, an idiopathic model of ASD, found that treatment with a non-specific, muscarinic cholinergic receptor agonist reduced repetitive behaviors including self-grooming and marble burying. Unknown is whether targeting specific muscarinic receptor subtypes would be sufficient to attenuate repetitive behaviors in BTBR mice. Treatment with a partial M_1 muscarinic receptor agonist was shown recently to improve behavioral flexibility in rats. Thus, targeting M_1 muscarinic receptors may be effective in reducing behavioral flexibility deficits and increased stereotyped repetitive behaviors in BTBR mice. The present study tested whether treatment with the partial M_1 muscarinic cholinergic receptor agonist, CDD-0102A, was sufficient to alleviate a reversal learning deficit and elevated grooming behavior in BTBR mice. As observed previously, BTBR mice exhibited elevated grooming and a reversal learning deficit compared to that of B6 mice. CDD-0102A at a 0.01 or 0.06 mg/kg dose (i.p.) did not attenuate elevated grooming behavior in BTBR mice. In contrast, CDD-0102A at 0.06 mg/kg did attenuate a reversal learning deficit in BTBR mice by reducing regressive errors. These findings suggest that treatment with an M_1 muscarinic cholinergic receptor agonist may be effective in treating cognitive flexibility deficits in ASD, but may not be effective in treating stereotyped repetitive behaviors.

Disclosures: **M.E. Ragozzino:** None. **H. Ramirez:** None. **J.T. Dunn:** None. **W.S. Messer:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: JSPS Res Fellow (PD 26-10961)

Intramural Res Grant (26-9) of NCNP

COI Stream Project (MEXT) for MRI device

Brain/MINDS Project (AMED)

Title: Atypical behavioral and neural phenotypes in a non-human primate model of autism spectrum disorders

Authors: *K. MIMURA[1,2,3], C. SATO[2], K. NAKAGAKI[1], I. AOKI[2], T. MINAMIMOTO[2], N. ICHINOHE[1,2];
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Abstract: Autism spectrum disorder (ASD) is a group of behaviorally defined neurodevelopmental disorders associated with social and cognitive disablities. In rodents, valproic acid (VPA) exposure *in utero* is widely used to an ASD model (Shin, 2015) because of its obvious surface fidelity. VPA has been reported as a risk factor of ASD in human clinical studies (Christensen, 2013). We reported VPA exposed ASD model in non-human primate, common marmoset (*Callithrix jacchus*) focused on abnormal psychiatric behavior in adulthood (Yasue, 2015). Here we show brain structural and behavioral phenotypes in early developmental stage of this model.
VPA exposed models of marmoset were obtained from the dams, which recieved 7 oral administrations of VPA at 200 mg/kg.day from 60 to 66 after conception.
Brain imaging study was conducted using 19 neonatal marmosets (P2 days of age, 9 VPA exposed, 10 unexposed; UE). Diffusion tensor images (DTI) were acquired using a 7 Tesla MRI system (20 cm bore, Bruker Biospin, Germany) in the *ex vivo* neonatal brain (TR/TE = 6500/26.56 ms, FOV = 32 × 32 mm^2, matrix size = 192 × 192, slice thickness = 1.0 mm, b value = 1000 s/mm^2, 30-direction). The corpus callosum (CC) and the anterior commissure (AC) were detected at mid-sagittal plane, and their sizes were measured by using Canny edge detection algorithm. In the mid-sagittal plane, neonatal VPA marmoset brains showed tendency that the AC was smaller than unexposed group. There have been no reports describing neonatal CC/AC structures with ASD even if in animal models. But bilateral hemispheric connection of human ASD patients, except neonate, were pepeatedly exxamined and shown to be weaker than that of normally grown human.
Behavioral study was conducted using 14 young marmosets (P30-150 days of age, 7 VPA, 7 UE) and their parents. To evaluate the kinship social communication, family (one juvenile and its parents) vocalizations were recorded for 30 minutes in their home cage carried at shield room. Vocal spectrogram was used to define and count 7 call types. As a result, in VPA families, “trill” call frequency, supposed ‘felling affinity’, was significantly decreased especially in ranging period (around 90 days of age). So, it is suggested that such abnormality of affinitive kinship communication in early life event could be translatable index of social disability between model animal and human cases.

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Poster

391. Autism: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 391.10/B23

Topic: A.07. Developmental Disorders

Title: Neonatal RU-486 exposure; a gender specific animal model for the low maternal progesterone hypothesis of autism.

Authors: *H. GARMAN[1], J. KASS[2], S. KWON[3], R. MALSKY[3], C. INFANTINO[3], P. WHITAKER[4];
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Abstract: Progesterone is a sex hormone involved in maintaining a healthy pregnancy as well as having specific effects on the developing fetal brain. Low progesterone-associated obstetrical complications (bleeding, miscarriage and infertility) are increased in autism and thus it may be that the low progesterone itself is what also causes the brain changes which have been observed in autism. Males and females express progesterone receptors during different time periods in early development and if progesterone is involved, sexually dimorphic effects should be seen dependent on the critical periods when progesterone is active. Here, we test two models of low maternal progesterone, using the progesterone antagonist, RU-486 (40 mg/kg), and subcutaneous injections on postnatal days 1, 2 and 5 (**MPR**; associated with **M**ale **P**rogesterone **R**eceptor expression in the hypothalamus) or on postnatal days 7, 9 and 11 (**FPR**; associated with **F**emale **P**rogesterone **R**eceptor expression in hypothalamus). Sprague Dawley rats were divided into three groups (n=20 for each). Physical, repetitive, social, and motor development was observed. Early developmental markers showed a marginally delayed righting reflex for the MPR injection group compared to the control and FPR ($t = 1.82$, $p < 0.08$). Consistent with our pilot study, negative geotaxis was delayed on post-natal day 8 for both the MPR ($t = 2.31$, $p < 0.03$) and FPR groups, with the FPR group showing the most delay ($t = 4.67$, $p < 0.00$). Interestingly, only the MPR group displayed more unproductive motor movements on postnatal day 8 ($t = 2.08$, $p < 0.05$). Repetitive behaviors were increased in females from the FPR group ($t = 3.13$, $p < 0.01$) and males from the MPR group compared to their respective control ($t = 2.23$, $p < 0.05$). Deficits in social bonding with the mother were found in the females of the FPR group compared to the control group ($t = 3.33$, $p < 0.01$). Interestingly, the males in the MPR group showed more irregular maternal bonding behavior by leaving the mother more often than the control group (this was not seen in the FPR group, $t = 2.71$, $p < 0.01$). In a social choice paradigm, males from the MPR group showed deficits in social contact compared to control ($t = 3.12$, $p < 0.01$), females from the FPR group showed marginal deficits in social contact compared to control ($t = 1.91$, $p < 0.09$). Our results show that maternal progesterone at critical periods contributes to the

gender differences of ASD. These results suggest a disruption in the neuroendocrine system leading to changes in physical development, motor, social and repetitive behavior.

Disclosures: H. Garman: None. **J. Kass:** None. **S. Kwon:** None. **R. Malsky:** None. **C. Infantino:** None. **P. Whitaker:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: Japan Society for the Promotion of Science; KAKENHI (21659063, 25460099, 26293020, 26670122, 11J03070, 13J05359, 15H01288, 16K15126, 16K19183)

Japan Society for the Promotion of Science; Program for Circulation of Talented Researchers.

Dainippon Sumitomo Pharma Co., Ltd. (Japan); Neuropsychiatry Drug Discovery Consortium and Joint Research Fund.

Title: Continuous activation of dopaminergic system improves autism-related behavioral abnormalities in mice prenatally exposed to valproic acid.

Authors: *S. HASEBE[1], Y. HARA[2], M. HIGUCHI[2], Y. AGO[2], T. NAKAZAWA[1,2], H. HASHIMOTO[2,3], T. MATSUDA[2], K. TAKUMA[1,3];
[1]Grad. Sch. of Dent., Osaka Univ., Osaka, Japan; [2]Grad. Sch. of Pharmaceut. Sci., Osaka Univ., Osaka, Japan; [3]United Grad. Sch. of Child Develop., Osaka Univ., Osaka, Japan

Abstract: Dosing of valproic acid (VPA), a widely used antiepileptic drug, during pregnancy has been reported to increase the risk of autism spectrum disorders (ASD) in children. We have recently demonstrated that mice prenatally exposed to VPA on embryonic day 12.5 provide a suitable animal model to study ASD (Hara et al., J. Pharmacol. Sci., 2012; Kataoka et al., Int. J. Neuropsychopharmacol., 2013; Takuma et al., Pharmacol. Biochem. Behav., 2014; Hara et al., Behav. Brain Res., 2015). In the present study, we examined whether the attention deficit/hyperactivity disorder (ADHD) treatment drugs methylphenidate and atomoxetine, which increase the extracellular levels of dopamine and noradrenaline in the prefrontal cortex of mice (Koda et al., J. Neurochem., 2010), could alleviate ASD-like behavioral abnormalities and a decreases in cortical dendritic spine density in the mice prenatally exposed to VPA. Pregnant ICR mice were intraperitoneally injected with either 500 mg/kg of VPA or saline on day 12.5 of

gestation. After birth, the offspring were weaned, sexed, and caged in groups of 5-6 mice of the same sex at 3 weeks old. Although acute administration of methylphenidate and atomoxetine increased prefrontal dopamine and noradrenaline release in the prenatal VPA-exposed mice at 8 weeks of age, it did not affect social interaction deficits and recognition memory impairment in VPA-exposed mice. In contrast, chronic administration of methylphenidate (3 mg/kg) and atomoxetine (1 mg/kg) for 2 weeks alleviated the ASD-like behavioral abnormalities. The chronic administration of the drugs also improved the decrease in dendritic spine density in the prefrontal cortex of the prenatal VPA-exposed mice. The ameliorative effects by chronic administration of the ADHD drugs on behaviors and dendritic spine morphology were blocked by the dopamine-D_1 receptor antagonist SCH39166 or the dopamine-D_2 receptor antagonist raclopride, but not by the α_2-adrenoceptor antagonist idazoxan. These findings suggest that chronic administration of methylphenidate and atomoxetine improves abnormal behaviors and diminishes the reduction in spine density in the mouse model of ASD through continuous activation of dopaminergic system.

Disclosures: **S. Hasebe:** None. **Y. Hara:** None. **M. Higuchi:** None. **Y. Ago:** None. **T. Nakazawa:** None. **H. Hashimoto:** None. **T. Matsuda:** None. **K. Takuma:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: MOST104-2321-B-010-022

MOST104-2311-B-010-010-MY3

Title: Neonatal treatments with risperidone partially reverses aberrant striatal compartmentation and ultrasonic vocalizations in a mouse model of autism spectrum disorder

Authors: H.-Y. KUO, *F.-C. LIU;
Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Human studies have reported that children with maternal exposure to valproic acid (VPA) have a high risk of developing autism spectrum disorder (ASD). Animal studies have shown that maternally VPA-treated rat offspring develop ASD-like behaviors, including stereotypic and abnormal social behaviors that are related to striatal function. We have previously found abnormal striatal compartments in maternally VPA-treated mice (Kuo et al.,

2013). Risperidone, a dopamine D2 receptor antagonist, is a FDA-approved clinical drug for treating ASD patients. Here, we tested if risperidone could rescue defective striatal compartments and improve ASD-like symptoms in $VPA^{E12.75}$ mouse offspring. The VPA- and saline-treated mouse offspring were daily injected with risperidone or its vehicle from P0 to P7. The pups were then assayed for ultrasonic vocalizations (USV) at P8. Risperidone treatments significantly reversed the duration within each call in $VPA^{E12.75}$ mice compared to vehicle-treated $VPA^{E12.75}$ mice, but it had no effects on other USV features. Correlating with the improvement in USV duration by risperidone, an increase in MOR1-positive striosomal area in the rostral striatum was found in risperidone-treated $VPA^{E12.75}$ mice compared to vehicle-treated $VPA^{E12.75}$ mice. Risperidone did not alter MOR1-positive areas in the middle and caudal striatum. The reduction of MOR1-positive striosomes in $VPA^{E12.75}$ striatum was associated with a failure of aggregation $BrdU^{E12.75}$-labeled cells into striosomal patches. We found that the risperidone treatments were able to partially restore clusters of $BrdU^{E12.75}$ cells in $VPA^{E12.75}$ striatum compared to the vehicle-treated group, though the number of $BrdU^{E12.75}$-labeled cells was not changed. Taken together, our study suggests that the VPA-induced USV deficits are at least partially rescued by risperidone treatments, which is correlated with partial restoration of striosomal compartment. This work was funded by MOST104-2321-B-010-022, MOST104-2311-B-010-010-MY3.

Disclosures: H. Kuo: None. **F. Liu:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.13/B26

Topic: A.07. Developmental Disorders

Support: Institutional Grant FRF141314

Title: Autism-related behavior in juvenile and adult mice following perinatal antidepressant exposure

Authors: C. M. BOND, *N. S. WOEHRLE;
Psychology Dept., Wittenberg Univ., Springfield, OH

Abstract: Studies show an association between maternal antidepressant use during pregnancy and nursing and autism spectrum disorders in offspring (Croen et al., 2011, Rai et al., 2013). Thus, animal studies are needed to examine the role perinatal antidepressant exposure plays in the development of autism-related traits. The social symptoms of autism include deficient

reciprocal social interactions and reduced interest in novel social settings, and the presence of similar traits in mice can be measured by the three-chamber social test (TCST; Moy et al., 2004). Moreover, reduced non-selective attention is an associated feature of autism, and average rearing duration is thought to provide an index of non-selective attention in rodents (DeLorey et al., 2008, Aspide et al., 2000).
In this study, we perinatally exposed mice to a selective-serotonin reuptake inhibitor antidepressant by administering fluoxetine (18 mg/kg/day in the drinking water) to breeders from the time of pair-mating through weaning. We examined offspring as juveniles (3 weeks old) and adults (6 weeks old) for sociability and preference for social novelty behaviors in the TCST, and locomotor and rearing behaviors in the open field.
We found that male and female mice exposed to fluoxetine perinatally exhibit deficits in sociability and social novelty-seeking as juveniles, and these autism-like behaviors persist into adulthood. We also found that perinatal fluoxetine exposure produces hypoactivity and increases average rearing duration. Our findings suggest that perinatal fluoxetine exposure induces long-lasting social and attention deficits.

Disclosures: C.M. Bond: None. **N.S. Woehrle:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: a Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (15K09873)

Title: Autistic-like behaviors with hyperactivity in mice lacking kirrel3

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

Abstract: We have reported that a member of the immunoglobulin superfamily, kirrel3, is expressed in the brain, including the striatum, hippocampus, and cerebellum, using *in situ* hybridization histochemistry. Bhalla et al. (Am J Hum Genet 83: 703, 2008) have reported that a patient with severe intellectual disability shows *KIRREL3* mutation. Recently, a deletion of chromosome 11 that includes *KIRREL3* gene has been reported in a patient with autism spectrum

disorders (ASDs) associated with Jacobsen syndrome (Guerin et al., Am J Med Genet 158A: 2551, 2012), suggesting that *KIRREL3* gene is a candidate for ASDs. By whole-genome sequencing of monozygotic twins with ASDs, three de novo point mutations of *KIRREL3* gene have been identified (Michaelson et al., Cell 151: 1431, 2012). However, it remains unclear how the disruption of *kirrel3* gene causes these neurodevelopmental disorders.
To gain insights into the role of kirrel3 in the brain, we first investigated the localization of kirrel3 protein using immunohistochemistry. The localization of kirrel3 protein was observed in the hippocampal mossy fiber terminals and the cerebellar basket cell terminals as well as in the synaptic glomeruli of olfactory and accessory olfactory bulb. These results suggest that kirrel3 may be involved in the synapse formation and synaptic plasticity of the specific circuits, including the hippocampal and cerebellar circuits.
To further elucidate the role of kirrel3 in the brain, we generated *kirrel3*-knockout (*kirrel3*$^{-/-}$) mice and investigated their behavioral phenotype. In the 3-chamber social interaction test, *kirrel3*$^{-/-}$ mice displayed normal social interaction, but impairment of social novelty. Social communication by ultrasonic vocalization was reduced in adult *kirrel3*$^{-/-}$ mice. In the open field test, *kirrel3*$^{-/-}$ mice showed increased locomotor activity and stereotyped peripheral circling. In the acoustic startle test, enhanced startle response to acoustic stimuli was observed in *kirrel3*$^{-/-}$ mice. Thus, *kirrel3*$^{-/-}$ mice exhibited the communication/social deficits, stereotyped behavior, and sensory abnormality (auditory hyperreactivity) in addition to the hyperactivity behavior, which are relevant to ASDs with attention deficit hyperactivity disorder.

Disclosures: **T. Hisaoka:** None. **T. Komori:** None. **H. Gyobu:** None. **T. Kitamura:** None. **Y. Morikawa:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.15/C2

Topic: A.07. Developmental Disorders

Support: Missouri State University Faculty Research Grant F07251-162001-022

Title: Stereological investigation of the rat thalamic nuclei following developmental hyperserotonemia.

Authors: *L. HOUGH, R. FOREMAN, C. GRUBB;
Biomed. Sceinces, Missouri State Univ., Springfield, MO

Abstract: Elevated blood serotonin in perinatal development (Developmental Hyperserotonemia) is the most consistent neurochemical finding reported in Autism Spectrum Disorder (ASD), and has been implicated in the pathogenesis of the disorder. Accordingly, pre- and postnatal administration of the non-selective serotonin agonist, 5-methoxytptamine (5-MT), has been hypothesized as a model of developmental hyperserotonemia (DHS) to investigate the behavioral and morphological implications in ASD. Our previous study, examining the effects of DHS found significant neurodevelopmental changes in the dendritic architecture and synaptic connectivity of neurons in the dentate nucleus of the cerebellum. The present investigation has gone further to describe alterations in the development of the dentate-thalamo-cortical pathway, a neural network involved in motor learning, automaticity of movements, and higher cognitive functions, shown to be affected in ASD. Using unbiased stereological techniques, serial sections of DHS rats were compared to age-matched controls, specifically analyzing the effects of treatment on nuclear volume, and estimated cell number, area, distribution, and volume within the principle (relay) nuclei of the thalamus. Results did not show a change in the overall volume of the thalamus or the principle nuclei. However, there were significant differences in the relationship between thalamic volume and total brain volume (TBV). When grouped by estimated TBV, mean thalamic volume was significantly reduced in the DHS group relative to controls. Additionally, significant reductions in cell numbers, density and distribution were observed in some subdivisions of the principle nuclei including the ventral anterior (VA), ventral lateral (VL), ventral posterolateral (VPL), and ventral posteromedial (VPM) nuclei. The thalamus forms a key component of neural systems, with reciprocal connections to virtually every major region of the brain. Alterations in these connections and neuronal organization may be implicated in the neuropathological and behavioral changes observed in ASD.

Disclosures: **L. Hough:** None. **R. Foreman:** None. **C. Grubb:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.16/C3

Topic: A.07. Developmental Disorders

Title: Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting from maternal immune activation in mice

Authors: ***M. J. WEISER**[1], B. MUCHA[2], H. DENHEYER[2], D. ATKINSON[2], N. SCHANZ[2], E. VASSILIOU[3], R. H. BENNO[2];

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Abstract: The prevalence of autism spectrum disorders over the last several decades has risen at an alarming rate. Factors such as broadened clinical definitions and increased parental age only partially account for this precipitous increase, suggesting that recent changes in environmental factors may also be responsible. One such factor could be the dramatic decrease in consumption of anti-inflammatory dietary omega-3 (n-3) polyunsaturated fatty acids (PUFAs) relative to the amount of pro-inflammatory omega-6 (n-6) PUFAs and saturated fats in the Western diet. Docosahexaenoic acid (DHA) is the principle n-3 PUFA found in neural tissue and is important for optimal brain development, especially during late gestation when DHA rapidly and preferentially accumulates in the brain. In this study, we tested whether supplementation of a low n-3 PUFA diet with DHA throughout development could improve measures related to autism in a mouse model of maternal immune activation. We found that dietary DHA protected offspring from the deleterious effects of gestational exposure to the viral mimetic polyriboinosinic-polyribocytidilic acid on behavioral measures of autism and subsequent adulthood immune system reactivity. These data suggest that elevated dietary levels of DHA, especially during pregnancy and nursing, may help protect normal neurodevelopment from the potentially adverse consequences of environmental insults like maternal infection.

Disclosures: **M.J. Weiser:** A. Employment/Salary (full or part-time): DSM. **B. Mucha:** None. **H. Denheyer:** None. **D. Atkinson:** None. **N. Schanz:** None. **E. Vassiliou:** None. **R.H. Benno:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.17/C4

Topic: A.07. Developmental Disorders

Support: R21 MH 104800

Title: Valproic acid induction of nrf2 in fetal but not adult brain

Authors: *J. GIFFORD[1], S. NORTON[2], A. KUSNECOV[2], G. C. WAGNER[2];
[1]Rutgers Univ., Piscataway, NJ; [2]Psychology, Rutgers Univ., New Brunswick, NJ

Abstract: Autism is associated with high levels of oxidative stress biomarkers, increased body burdens of environmental toxicants that induce oxidative stress, and decreased levels of

antioxidants. A master regulator for the induction of the antioxidant response is the transcription factor, NRF2. Here we measured NRF2 expression as an indicator of an oxidative stress response following administration of valproic acid (VPA) to pregnant C57BL/6 mice. In adults, VPA is used as a GABAergic anticonvulsant and mood stabilizer. However, exposure *in utero* to VPA results in an autism-like syndrome. This differential response has been attributed to the GABA receptor activation being excitatory during early development, after which there is a shift to its recognized inhibitory function. This GABAergic shift in action is predicted to result in a differential oxidative stress response in the embryo (excitatory) relative to the dam (inhibitory). VPA (300 mg/kg, s.c.) was administered to pregnant mice on E12.5 and brain tissue was examined 2 hours later to determine if NRF2 protein expression was altered in either the dam and/or the embryos. Tissue was extracted and protein separated using SDS-PAGE gels. The membrane was then probed for NRF2and α-tubulin (Cell Signaling Technologies). Results were expressed as protein target ratio to α-tubulin. It was found that VPA increased NRF2 protein levels in the embryonic brains, but not in the brains of dams. The induction of embryonic NRF2 is likely a protective mechanism to defend against oxidative stress and suggests that VPA may be a risk factor for neurodevelopment during the prenatal period at a time when the GABAergic system is excitatory. This is consistent with previous data from our laboratory that showed VPA treatment altered a variety of behaviors that mark normal developmental milestones. This included alterations in motor reflexes and social investigation. Further, the toxicity exerted by VPA in the embryo may be through excitotoxic-engendered oxidative stress mechanisms.

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Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: Sigma Xi

Title: The effects of fastigial nuclei inactivation on social behavior in the rat

Authors: *V. K. BEHNKE, M. E. STEVENSON, H. E. HOBSON, J. R. KRUEGER, V. G. BELTRONE, A. S. NAZARIO, R. A. SWAIN;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Autism and autism spectrum disorders are developmental disabilities caused by brain abnormalities. These disorders affect more than one percent of the population (Minshew &

Williams, 2007). Recent research has implicated the cerebellum and its deep nuclei in autism. The dentate nuclei function in spatial processing and motivation (Joyal, Strazielle, & Lalonde, 2001), and the interpositus nuclei facilitate conditioning and learning (Christian & Thompson, 2003). The function of the fastigial nuclei, however, is more ambiguous. The present study examined the fastigial nuclei's role in mediating social interaction. The experiment questioned whether damage to the fastigial nuclei in rats would result in behaviors that could model the abnormal social behaviors seen in people with autism. Bilateral cannulation surgery was performed on 15 Long-Evans hooded rats. A within-subjects ABABAB reversal design was implemented. All animals received a microinfusion of saline during the A phases as baseline measures for social interaction. Social interactions were tested using a unique social interaction chamber and an open field with a confederate animal simultaneously occupying the apparatus. During the treatment phase, half of the animals received microinfusions of the anesthetic drug bupivacaine, which temporarily inactivated the fastigial nuclei. The other half received saline again and served as a control group. Social interaction was tested again for the treatment phase. This sequence was executed three times over a total of six days to achieve an ABABAB design. Social contact between the experimental and confederate animals was measured using multiple dependent variables, which allowed for delineation of the type of contacts in which the animals engaged. Results showed no difference in quantitative social interactions, but showed numerous significant differences in qualitative social interaction. Animals with inactivated fastigial nuclei engaged in more contacts with their body, but fewer contacts using their nose. This suggests animals with inactivated fastigial nuclei engaged in less intense and more passive social interactions. These animals also engaged in more behaviors to prevent social interaction. Similarly, people with autism engage in less intense, more passive, and more preventative behaviors than people without autism. This suggests animals with inactivated fastigial nuclei model some of the abnormal social behaviors seen in autism. Knowledge that the fastigial nuclei mediate the quality of social interactions can lead to breakthroughs in autism treatments and further the understanding of pathology in the autistic brain.

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Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH training grant T32 007051

DOD W81XWH-13-0306

Title: Developmental exposure to the selective serotonin reuptake inhibitor citalopram alters spatial learning and memory, anxiety, sociability, and acoustic startle response in the offspring of Sprague-Dawley rats as adults

Authors: *J. NELMS SPROWLES[1], J. R. HUFGARD[2], A. GUTIERREZ[2], R. A. BAILEY[2], S. A. JABLONSKI[1], M. T. WILLIAMS[1,2], C. V. VORHEES[1,2];
[1]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Col. of Med., Univ. of Cincinnati, Cincinnati, OH

Abstract: Depression is widespread, and women are more likely to be affected, with the highest rate of occurrence during child-bearing years. Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed to women during pregnancy and the postpartum period. Thus, thousands of children are exposed to these serotonergic drugs *in utero* each year. SSRIs block the serotonin reuptake transporter (SERT) to increase synaptic 5-HT. Therefore, SSRIs may interfere with normal serotonergic modulation of development and impact brain ontogeny and behavior. Epidemiological studies report increased prevalence of autism spectrum disorder (ASD) in children whose mothers took SSRIs while pregnant (Boukhris et al., 2016; Croen et al., 2011). Studies in rodents have reported increased depression and anxiety-related behavior, decreased aggression and sociability, and impaired cognition following developmental SSRI exposure. The present study investigated whether pre- and neonatal exposure to citalopram influences behavioral outcomes. Sprague-Dawley dams were assigned to one of two treatment groups: Citalopram (10 mg/kg; CIT) or Saline (Sal). Dams were treated by subcutaneous injection twice daily (6 h apart) from E6-21, and pups were dosed from P1-20. Behavioral testing began on P60. One male/female pair from each litter was tested in the Cincinnati water maze for egocentric learning. Another pair received the Morris water maze to assess allocentric learning and memory. A third pair received elevated zero maze, open-field, marble burying, acoustic startle response with prepulse inhibition (PPI), social preference, and forced swim. There was no effect of treatment on swimming ability. Analyses showed significant treatment effects in Morris water maze acquisition (CIT rats had reduced path efficiency than Sal rats, $p \leq 0.01$), reduced open-field exploration ($p \leq 0.05$), increased marble burying ($p \leq 0.001$), increased PPI ($p \leq 0.01$), and reduced social preference ($p \leq 0.05$), but no changes in egocentric learning. The results suggest that developmental SSRI exposure results in enduring changes in behavior in the adult offspring consistent with an ASD-like phenotype.

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Poster

391. Autism: Models

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University of California Davis MIND Institute

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Title: GABA-B receptor agonist r-baclofen reverses behavioral deficits in 16p11.2 deletion mice

Authors: *M. SCHAFFLER[1], T. M. KAZDOBA[2], J. N. CRAWLEY[2];
[1]Psychiatry & Behavioral Sci., UC Davis, Sacramento, CA; [2]Psychiatry & Behavioral Sci., UC Davis MIND Inst., Sacramento, CA

Abstract: Deletion of chromosomal region 16p11.2 has been reported in approximately 0.6% of autism cases (Weiss et al., 2008; Hanson et al., 2010). 16p11.2 deletion syndrome is associated with developmental delay, intellectual disability, macrocephaly, obesity, language impairments and social deficits (Hanson et al., 2010; Shiwany et al., 2010; Hanson et al., 2015). R-baclofen, a GABA-B receptor agonist, was previously found to improve social and cognitive deficits in the *Fmr1* mouse model of Fragile X syndrome, (Henderson et al., 2012; Qin et al., 2015), and to reduce repetitive self-grooming and improve sociability in the BTBR mouse model of autism (Silverman et al., 2015). To evaluate the behavioral effects of r-baclofen in 16p11.2 deletion mice, we administered r-baclofen chronically in home-cage drinking water (0.5 mg/ml) to the Dolmetsch line of 16p11.2 heterozygous deletion mice and to their wildtype littermates. Control mice of each genotype received untreated drinking water. Subject mice were housed by sex in mixed genotype cages. To evaluate cognitive ability, we conducted the object location memory task using standard methods, with a one hour interval between habituation and testing as previously described (Yang et al., *Learning and Memory*, 2015). 16p11.2 heterozygotes failed to display significant object location memory. R-baclofen reversed this cognitive deficit. No differences in exploratory activity were detected during the habituation session. To evaluate sociability, we tested male-female reciprocal social interactions, as we had previously found that 16p11.2 male mice displayed fewer interactions with an estrous C57BL/6J female on some parameters (Yang et al., *Autism Research*, 2015). Videos were manually scored for male-female social interactions. Preliminary data suggest that r-baclofen treatment improved some parameters of social behaviors in 16p11.2 heterozygote males during male-female interactions. These findings support the hypothesis that Arbaclofen may offer an effective therapeutic for treating

some of the symptoms of 16p11.2 deletion syndrome (Berry-Kravis et al., 2012; Erickson et al., 2014).

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Poster

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Topic: A.07. Developmental Disorders

Support: NIH T32MH073124-06

International Rett Syndrome Foundation 5R01NS081913-14

Title: The impact of maternal care on a female mouse model of Rett syndrome

Authors: *A. VOGEL CIERNIA[1], M. PRIDE[2], A. NORONHA[2], A. CHANG[2], D. YASUI[2], J. N. CRAWLEY[2], J. M. LASALLE[2];
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Abstract: Rett syndrome is a neurodevelopmental disorder caused by mutations in the gene encoding methyl CpG binding protein 2 (MeCP2) that occur in 1:10,000 female births. Rett syndrome is characterized by a period of normal development followed by regression at 6-18 months of age and the onset of severe motor impairments. *Mecp2* mutations in mice recapitulate many of the clinical features of Rett syndrome, but the majority of behavioral assessments have been conducted in male MeCP2 hemizygous null mice. Given that Rett syndrome is predominately found in girls, we assessed heterozygous *Mecp2* mutant female mice (*Mecp2*$^{-/+}$) in a behavior battery that included measures of anxiety (elevated plus maze, light-dark box), activity (open field), motor function (gait analysis, accelerating rotarod, beam walking), sociability (three chamber social approach), and cognition (object location and recognition memory). Prior work with *Mecp2*$^{-/+}$ mice suggested that symptom onset was delayed until 6-9 months of age; however, we observed deficits in motor behaviors and alterations in activity much earlier (6-7 weeks of age). In comparison, sociability and short-term memory were intact in the *Mecp2*$^{-/+}$ mice, suggesting that the motor impairments were the predominant phenotype of this model. We also assessed the impact of maternal care on behavioral outcomes in MeCP2$^{-/+}$ mice, since breeding necessitates *Mecp2*$^{-/+}$ dams that have known adverse maternal behaviors. Cross fostering within 24hrs of birth to a CD1 dam resulted in litters with significantly higher body weights post weaning independent of genotype, and surprisingly, the fostered MeCP2$^{-/+}$

mice continued to gain weight into adulthood at a rate significantly surpassing their wildtype littermates and non-fostered *Mecp2*$^{-/+}$ mice. The larger body weight of the fostered MeCP2$^{-/+}$ mice may have further impaired performance on several of the motor tasks. Future work will be needed to longitudinally examine the relationship between maternal care, body weight, and motor task performance in MeCP2$^{-/+}$ mice. Overall, our findings indicate that MeCP2$^{-/+}$ mice recapitulate many of the motor aspects of Rett syndrome earlier than previously appreciated and that environmental factors, such as maternal care, may impact the phenotype severity.

Disclosures: **A. Vogel Ciernia:** None. **M. Pride:** None. **A. Noronha:** None. **A. Chang:** None. **D. Yasui:** None. **J.N. Crawley:** None. **J.M. LaSalle:** None.

Poster

391. Autism: Models

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Program#/Poster#: 391.22/C9

Topic: A.07. Developmental Disorders

Support: NIH Grant 5T32MH074249

Title: Correction of cognitive and behavioral deficits in a 16p11.2 CNV mouse model by selective activation of $GABA_B$ receptors with r-baclofen

Authors: *L. J. STOPPEL, A. R. PREZA, A. J. HEYNEN, M. F. BEAR;
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Abstract: Human chromosome 16p11.2 microdeletion is the most common gene copy number variation (CNV) in autism, affecting an estimated 3 in 10,000 people. Caused by a single copy deletion of ~27 genes, people with 16p11.2 microdeletion syndrome often exhibit impaired communication and socialization skills as well as intellectual disability. Studies on animal models of human 16p11.2 microdeletion disorder have revealed morphological, behavioral and electrophysiological deficits. Moreover, recent evidence suggests that neuronal dysfunction downstream of metabotropic glutamate receptor 5 ($mGlu_5$) contributes to cognitive deficits in mouse models of 16p11.2 microdeletion disorder and may be a point of convergence in the synaptic pathophysiologies of this and other genetic causes of autism including Fragile X Syndrome. Recently, R-baclofen, a $GABA_B$ receptor agonist, was shown to correct many disease-related pathologies in a mouse model of Fragile X Syndrome including cognitive impairments, and has shown promising outcomes in clinical trials with patients with autism and Fragile X Syndrome. We investigated the efficacy of r-baclofen to treat cognitive deficits in heterozygous 16p11.2 deletion mice generated by Alea Mills' laboratory. We administered r-

baclofen chronically in the drinking water (0.5 mg/ml) in 8-12 week old group-housed heterozygous (df/+) and wild-type male littermates for 2 weeks prior to behavioral testing. We found that vehicle-treated df/+ mice exhibited profound deficits in a familiar object recognition task and contextual fear discrimination task, similar to previous reports. Additionally, we found that df/+ mice exhibit hyperactivity, spend more time in the center in the open field and fail to habituate across testing sessions. Importantly, chronic r-baclofen treatment corrected deficits in object recognition and contextual fear discrimination and restored normal habituation and behavior in the open field. Our results indicate that inhibition of $GABA_B$ receptors with r-baclofen may be a promising treatment avenue for patients with 16p11.2 microdeletion disorder and other genetic causes of autism with similar dysfunction.

Disclosures: **L.J. Stoppel:** None. **A.R. Preza:** None. **A.J. Heynen:** None. **M.F. Bear:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: IDDRC Grant U54 HD079125

Title: Testing two mouse models of 16p11.2 deletion syndrome in touchscreen learning and reversal tasks

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Abstract: Recurrent heterozygous deletions of a ~600kb segment on human chromosome 16 is found in ~0.4% of individuals with intellectual disabilities (ID) and 0.6% of cases of autism (Weiss et al., 2008; Hanson et al., 2015). 16p11.2 deletion is also associated with speech delay, developmental delays, psychiatric disorders, and obesity. The present study evaluated associative learning and cognitive flexibility in two mouse models of 16p11.2 deletion, the Dolmetsch, generated at Stanford University in the Dolmetsch Lab (Portmann et al., 2014), and the Mills line, generated by the Mills' Lab at Cold Springs Harbor (Horev et al., 2011). Previous studies reported impaired novel object recognition in heterozygous (+/-) mice of both lines (Portmann et al., 2014; Pucilowska et al., 2015). Impaired object location memory and social memory were detected in Dolmetsch +/- (Yang et al., 2015a). Deficits in contextual fear conditioning and passive avoidance were reported for the Mills line (Tian et al., 2015), but not in the Dolmetsch

line (Yang et al., 2015b). In this study we used the automated Bussey-Saksida touchscreen system for mice (Campden Instruments Ltd/Lafayette Instruments, Lafayette, IL, USA) to further examine cognitive phenotype deficits in both lines. We evaluated the basic pairwise visual discrimination and reversal learning tasks, as described previously (Silverman et al., 2015; Yang et al., 2015b). To further explore cognitive flexibility, we also pioneered a re-reversal task. In pre-training stages, there were no genotype differences in days to reach criterion for the Dolmetsch line. Similarly, +/- of the Mills line exhibited no deficits in pre-training. In the pairwise visual discrimination test, deficits were replicated in the Dolmetsch line +/- (Yang et al., 2015b), but were not detected in Mills +/-. In our novel re-reversal task, correct and incorrect images were again switched. All Dolmetsch wildtype (+/+) mice were able to reach criterion, whereas all but one +/- failed the reversal task and were not eligible for the re-reversal training. In comparison, all +/+ and +/- of the Mills line were advanced to, and were able to complete, the re-reversal task. In summary, we replicated pairwise visual discrimination impairments and cognitive inflexibility in the Dolmetsch +/-. Preliminary testing of the Mills line revealed no apparent deficits in pairwise discrimination, reversal, or re-reversal training. Future experiments will explore other aspects of cognitive functions, using transitive inference and paired-association learning.

Disclosures: **B.L. Onaga:** None. **G. Liow:** None. **M.S. Sarvi:** None. **M. Yang:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: T32MH073124

Title: Evaluation of the TrkB agonist 7,8-dihydroxyflavone in the BTBR mouse model of autism

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Abstract: Dysregulation of brain-derived neurotrophic factor (BDNF) signaling is a possible contributing factor in autism spectrum disorders. BDNF, a member of the neurotrophin family of growth factors, is crucial for the growth and differentiation of new synapses and neurons as well as neuronal survival. BTBR T+tf/J (BTBR) mice, an inbred mouse strain that models idiopathic autism, display impaired sociability, excessive grooming and cognitive deficits (McFarlane et al.,

2008; Silverman et al., 2013; Yang et al., 2012). Reductions in BDNF signaling and mRNA, as well as levels of the BDNF TrkB receptor, have been identified in several brain regions in BTBR mice, as compared to the social strain C57BL/6 (B6) (Stephenson et al., 2011; Scattoni et al., 2013; Jasien et al., 2014). In the current set of studies, subchronic administration of the TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF), was evaluated in BTBR and B6 mice to determine its effects on sociability and repetitive behaviors. Administration of 7,8-DHF was not detrimental to B6 social behaviors, as assessed by the 3-chambered social approach task and male-female reciprocal social interactions. BTBR mice administered 7.5 mg/kg 7,8-DHF i.p. spent significantly more time with the novel social mouse than with the novel cup. In addition, BTBR mice administered 2.5, 5, or 7.5 mg/kg 7,8-DHF spent significantly more time sniffing the novel social mouse compared to the novel cup. 7,8-DHF did not improve BTBR social behaviors in the male-female reciprocal social interaction task. 7,8-DHF did not have any significant effects on B6 or BTBR repetitive behaviors, and did not significantly affect open field locomotion. These data suggest that modulation of the BDNF signaling pathway may have modest effects on sociability.

Disclosures: **T.M. Kazdoba:** None. **P.T. Leach:** None. **K. Sison:** None. **J.N. Crawley:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant NS085709

NIH Grant MH073124

Title: Touchscreen visual discrimination learning and water maze deficits in the Ts65Dn mouse model of Down syndrome

Authors: *P. T. LEACH[1,2], T. M. KAZDOBA[2], K. SISON[2], C. M. GALL[3], G. LYNCH[4], J. N. CRAWLEY[2];
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Abstract: Down syndrome, a leading genetic cause of intellectual disabilities, is caused by a trisomy of chromosome 21. Mouse studies of Down syndrome have frequently used the

homologous Ts65Dn mouse model, which displays deficits on some cognitive tasks including Morris water maze, fear conditioning, and novel object recognition (Costa, Stasko, Schmidt, & Davisson, 2010; Holtzman et al., 1996; Lockrow, Boger, Bimonte-Nelson, & Granholm, 2011). We tested cortically-dependent learning in Ts65Dn mice with a novel touchscreen-based operant task. Touchscreen cognitive assays are frequently used in human cognition testing, offering face validity and potential translational value for testing pharmacological and behavioral interventions. Food restricted WT and Ts65Dn male and female mice were trained on acquisition of a pairwise visual discrimination task, in which an X symbol and an = symbol of matched illumination were presented simultaneously on a touchscreen located at the front panel of the chamber. Image location alternated randomly between left and right image locations. Mice were pseudorandomly assigned to be rewarded for pressing the X versus the = image. Pressing the correct image was reinforced with 20 µl strawberry Ensure milkshake, while incorrect image touches were followed by a 20 sec timeout. Survival curves and unpaired t-tests revealed genotype differences on acquisition of the visual discrimination task. Ts65Dn took significantly more trials to reach criterion than WT (p=.01), and showed a strong trend for more days to reach criterion (p=.06) while there were no differences in the number of trials completed per day or on pre-training performance. Further, in a separate cohort of Ts65Dn and WT mice, we replicated Morris water maze spatial navigation deficits in learning the hidden platform location, confirming previous reports. Acquisition of the visible platform using proximal cues did not differ between genotypes, confirming normal visual and swimming abilities. These studies extend the range of cognitive phenotypes in the Ts65Dn mouse model of Down syndrome to a touchscreen assay with procedural methodologies analogous to standard human cognitive tests.

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Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant NS092216

Title: Forebrain loss of active Met tyrosine kinase disrupts cortical connectivity and GABA signaling

Authors: S. TANG[1], S. XU[2], W. ZHU[2], F.-S. LO[3], R. S. ERZURUMLU[3], *E. M. POWELL[4]; [1]Grad. Program in Toxicology, [2]Diagnos. Radiology and Nuclear Med., [3]Anat. & Neurobio.,

Univ. of Maryland Sch. of Med., Baltimore, MD; [4]Anat/Neurobio, Univ. Maryland, Baltimore, Baltimore, MD

Abstract: *MET*, the gene encoding the tyrosine kinase receptor for hepatocyte growth factor/scatter factor (HGF), has been identified as a susceptibility locus for autism spectrum disorders (ASD). Genetically altered mice with a targeted kinase-dead *Met* expressed in the cerebral cortex and hippocampus (under the control of the *Emx1-cre* driver), offer a useful model for the human susceptibility alleles of *MET*. Our previous structural magnetic resonance imaging (MRI) studies demonstrated an expansion of the sensory, motor and frontal cortical regions. We report resting state functional MRI with the graph-analysis toolbox with 37 regions of interest defined for the mouse brain, to examine neuronal connectivity in heterozygous *Met-Emx1,* and fully inactive homozygous *Met-Emx1* mice, and controls. Global network measures, including small-world index, clustering coefficiency, local efficiency, transitivity, assortativity, and modularity, were significantly lower in heterozygous *Met-Emx1* mice compared to control mice. Regional measures of clustering coefficient, local efficiency, degree, and node betweenness were altered in both heterozygous and homozygous *Met-Emx1* mice compared to control animals. The changes indicated greater local connectivity and reduced long range interactions in *Met-Emx1* brains, consistent with reports in individuals with ASD and those harboring *MET* autism susceptibility alleles. Behavioral observations also indicate potential altered sensory processing in *Met-Emx1* mice. Imbalances in the ratio of excitation to inhibition have been cited as an underlying cause of autism and a phenotype in multiple animal models of ASD. Electrophysiology of layer IV somatosensory cortical neurons from *Met-Emx1* brains indicated reduced inhibition. Attempts to increase inhibition by recruiting additional GABA-A receptors to the neuronal membrane were unsuccessful in *Met-Emx1* cortex. The lack of sufficient GABA-A receptors led to the hypothesis of increased free extracellular GABA concentration. In vivo magnetic resonance spectroscopy (MRS) measurements of neurotransmitters demonstrate significant increased GABA concentration in male *Met-Emx1* mice, with no changes in female *Met-Emx1* mice. The MRS data support a new role for Met in regulating GABA concentration and availability in a sex-dependent manner.

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Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH NS066392

NIH AT008742

NIH NS065957

Title: Ketogenic diets improve behaviors associated with autism spectrum disorder in the EL mouse

Authors: *D. N. RUSKIN, J. A. FORTIN, S. BISNAUTH, S. A. MASINO;
Trinity Col., Hartford, CT

Abstract: Attempts to ameliorate the core symptoms of autism spectrum disorder (ASD) with medications have had little success. Symptoms of ASD are frequently comorbid with a diagnosis of epilepsy and vice versa. Severe drug-refractory cases of comorbid ASD and epilepsy can have very poor outcomes, even with epilepsy surgery. Ketogenic diets (KDs) are remarkably effective treatments for epilepsy, and there is accumulating evidence for beneficial effects of ketogenic diets against core symptoms of ASD in animal models and patients. This study tests the behavioral effects of KD feeding in a murine model of comorbid ASD and epilepsy, the EL mouse strain. EL mice were fed control diet or one of two KD formulas ad libitum starting at 5 wk of age. Beginning at 8 wk of age, diet protocols continued and performance of each group on tests of sociability and repetitive behavior was assessed. Behavior was never worsened by a KD; results were mixed and generally positive depending on sex and type of test. KD feeding improved sociability and repetitive behavior under some conditions in female mice. In males, KD feeding did not significantly improve sociability, but did reduce self-directed repetitive behavior in a single-chamber test. Experiments with KDs of differing strength in female mice showed that a less strict, more clinically-relevant diet was fully effective. Behavioral improvements did not depend on a significant decrease in blood glucose; they might depend on ketosis although ketosis alone was not sufficient. Taken together these results add to the growing number of studies suggesting that KDs and related diets may provide significant relief from the core symptoms of ASD, and suggest there may be increased efficacy in females.

Disclosures: **D.N. Ruskin:** None. **J.A. Fortin:** None. **S. Bisnauth:** None. **S.A. Masino:** None.

Poster

391. Autism: Models

Location: Halls B-H

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Program#/Poster#: 391.28/C15

Topic: A.07. Developmental Disorders

Title: Ketogenic diet improves decreased mitochondrial respiration and activities of electron transport chain complex I and pyruvate dehydrogenase in BTBR autistic mice

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Abstract: Autism spectrum disorder (ASD) is a highly prevalent neurodevelopmental condition (1/68 in United State) that is characterized by specific behavioral and cognitive impairments, and is believed to arise from heterogeneous causes. Although a single and unifying pathophysiological mechanism is unlikely, there is growing evidence that mitochondrial dysfunction may play a critical role in provoking behavioral symptoms.
The BTBR T+tf/J (BTBR) mouse is a robust model of ASD and displays all three core behavioral features compared to C57 mice. Earlier, it was shown that the ketogenic diet (KD) can reduce autistic behaviors in BTBR mice (PMID: 23755170).
Here, we hypothesize that impaired mitochondrial bioenergetics may contribute to the etiology of ASD and that behavioral improvements induced by the KD are associated with changes in mitochondrial metabolism. To demonstrate this, we measured the mitochondrial oxygen consumption rate (OCR) using the Seahorse Bioscience XF24 analyzer. We observed significantly lower OCR levels in BTBR mice than in C57 control mice ($p<0.001$, N=13/group), and the KD improved OCR levels in BTBR mice ($p<0.05$, N=13/group). To determine the underlying mechanism for the observed decreased mitochondrial respiration in BTBR mice, we measured the activities of two of the five mitochondrial electron transport chain (ETC) complexes (Complex I & II), and pyruvate dehydrogenase (PDH). The activity of complex I was significantly decreased in autistic mice compared to control mice ($p<0.05$, N=4~5/group) and PDH activity showed the attenuated trend but was not significant ($p=0.073$, N=4~5/group). The KD led to statistically-significant improvements in the BTBR mice ($p<0.05$ for complex I and $p<0.001$ for PDH). The activity of Complex II was higher in autistic mice than in C57 controls ($p=0.0562$, N=6/group) and was significantly attenuated by the KD ($p<0.01$, N=6/group). Collectively, these observations suggest that mitochondrial dysfunctions such as dysregulation of mitochondrial respiration and abnormalities of Complex I, II and PDH in the brain may be important contributors to the autistic phenotype of BTBR mice, that the ketogenic diet can improve these mitochondrial dysfunctions in ASD, and that this putative improvement derived from better OXPHOS management may affect behavioral symptoms. Thus, we anticipate that this data may provide further evidence that abnormalities in mitochondrial function may underlie in part the behavioral features of ASD, and that the ketogenic diet may have potential therapeutic use.

Disclosures: Y. Ahn: None. **N. Yee:** None. **R. Tobias:** None. **J.M. Rho:** None.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Title: Identification of circuits regulating socially-directed behavior using DREADD-fMRI.

Authors: *M. BENEKAREDDY[1], T. J. STACHNIAK[2], M. VON KIENLIN[2], B. KUENNECKE[2], A. GHOSH[3];
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Abstract: Impairment in social function is one of the core deficits of autism spectrum disorder (ASD) and a prominent feature of schizophrenia (SZ). The neural circuit basis of impaired social function in disease states is only beginning to be understood with evidence pointing to an important role played by frontal cortical areas. In this study, using a combination of chemogenetics (Rogan and Roth, 2011), and rodent social behavior tasks, we assessed the consequences of altered neural activity in the prefrontal cortex on social behavior in rats and mice. To identify global circuit partners of the PFC and generate hypotheses about specific brain regions that act in tandem with the PFC in mediating social dysfunction, we performed a neuroimaging screen with magnetic resonance imaging (MRI) for brain-wide activity changes induced by the DREADD (Designer Receptors Exclusively Activated by Designer Drug) technology (Armbruster et al., 2007; Vardy et al., 2015). We further refined this functional PFC network by screening for co-regulations during bidirectional modulation of PFC activity. Acute activation of the prefrontal cortex using hM3D, the Gq coupled activating designer GPCR in excitatory neurons leads to a reversible decrease in sociability in both rats and mice. Chronic perturbation of PFC activity either by expressing hM3D in excitatory neurons or hM4D - the Gi coupled inhibitory designer GPCR - in parvalbumin or somatostatin inhibitory neuronal subtypes alters social function in a time-point and cell type dependent manner. In a further effort to investigate the brain-wide consequences of activating the prefrontal cortex, animals were subjected to MRI during acute activation of the prefrontal cortex. Prefrontal cortex activation led to a significant increase in blood perfusion in the prefrontal cortex (as expected), and led to the activation of brain regions like the nucleus accumbens, and dorsal raphe nucleus, known to be involved in the regulation of emotional behaviors. Our results lend further credence to the hypothesis that reciprocal connections between PFC and several key subcortical regions provide a circuit level framework for altered cortical and sub-cortical function to modify social behavior.

Disclosures: **M. Benekareddy:** A. Employment/Salary (full or part-time): F. Hoffman-La Roche. **T.J. Stachniak:** A. Employment/Salary (full or part-time): F. Hoffman-La Roche. **M. von Kienlin:** A. Employment/Salary (full or part-time): F. Hoffman-La Roche. **B. Kuennecke:** A. Employment/Salary (full or part-time): F. Hoffman-La Roche. **A. Ghosh:** A. Employment/Salary (full or part-time): E-Scape Bio.

Poster

391. Autism: Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant R00HD067379

Title: Digging behavior discrimination: a new test you will dig

Authors: H. L. POND, J. ADELMAN, O. MCKISSICK, *M. MANZINI;
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Abstract: The marble burying test, where digging is measured as related to the number of marbles buried in deep bedding, has become a popular assay for anxiety, obsessive compulsive disorder (OCD), and most recently, for repetitive behaviors in mouse models of autism spectrum disorder (ASD). While digging intensity is used to test repetitive behaviors and anxiety, digging is also a normal mouse behavior, that can be focused toward different goals: foraging for food, burrowing for shelter, removing noxious stimuli, or even for recreation, as has been shown for dogs and ferrets. The marble burying test originated as a test to analyze species-specific defensive behaviors in rats. However, rat behaviors do not necessarily translate to mice. Mice do not actively bury the marbles and do not spend much time interacting or reacting to the marbles at all, suggesting that the number of marbles buried is incidental to digging activity. Here, we present the Digging Behavior Discrimination test, a new paradigm to obtain clear distinctions between different types of digging behavior in mouse models based on the driving motivation for each type of digging. We look at food-seeking digging behavior as well as burrowing digging behavior, and how these related to the presence of marbles. To distinguish between food-seeking digging behavior and burrowing digging behavior we set up a clear cage with ample amount of corncob substrate. In one corner is a transparent tube that is filled with paper bedding. Mice are then placed into the apparatus and monitored for 30 minutes to observe their behavior. Wild-type mice fed ad libitum are compared with food-deprived animals following 20-25% weight loss. Satiated mice are more motivated to burrow in a new environment and spend significantly more time in the burrowing area than the food-deprived mice. In addition, the food-deprived mice

spend more time in the open area digging than they do in the burrow. To make sure the difference was not just due to acclimatization to a new environment, we performed the test again on the food deprived mice two weeks after their food intake returned to ad libitum. Once they were satiated again, the mice switched behaviors spending more time in the burrowing area. In general, the mice are indifferent to the presence or absence of marbles in the apparatus. This indicates that laboratory mice display digging behaviors with specific motivations, while they perform digging tests. This novel test could be used to better understand animal models of neuropsychiatric disorders to probe brain circuits involved in motivation, food seeking, shelter/safety seeking and anxiety.

Disclosures: **H.L. Pond:** None. **J. Adelman:** None. **O. McKissick:** None. **M. Manzini:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: Vassar College internal funds

Title: Delayed and reduced baseline and social isolation-potentiated ultrasonic vocalization in neonatal fragile X knockout mice

Authors: ***B. ZUPAN**[1], S. R. M. DEWIL[2], L. MORSE[2];
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Abstract: Silencing of the fragile X mental retardation (*FMR1*) gene causes Fragile X syndrome (FXS) and is the most common monogenic cause of autism. Symptoms shared by both neurodevelopmental disorders include cognitive inflexibility, hyperactivity and abnormal social behavior. Although the mouse model of FXS, the *fmr1* knock-out (KO) mouse, recapitulates a number of these symptoms, reports of abnormal social interaction and altered ultrasonic vocalization (USV) in both adult and neonate have been inconsistent. USV is an easily quantifiable measure of social communication and pup-dam interaction during early postnatal development, and abnormal vocalizations have been observed in mouse models of a number of neurodevelopmental disorders. Here we assessed USVs emitted by *fmr1* KO and wild-type (WT) neonate mice and found decreased call number and total call duration in both male and female KO but not WT pups at postnatal day (P) 4 and 8. Number of USVs did not change over time in WT mice, but KO pups showed an increase in vocalizations on P12 relative to P4 and 8, data suggestive of delayed onset of social communication in *fmr1* deficient pups. This interpretation

is further supported by the observation of alterated social isolation-potentiated vocalization (a modified maternal potentiation paradigm). Specifically, WT but not KO pups emitted increased number of calls following a five-minute social isolation at P4 and P12, while KO mice showed marginal potentiation at P8 only. Although maternal behavior can influence pup USVs, we observed no differences in maternal care between WT and KO dams. Taken together, our data suggest that the lack of *fmr1* may delay the temporal onset of social communication in the *fmr1* deficient neonate mouse and that this delay may be a marker of neurodevelopmental alterations underlying differences in adult social behavior. Indeed, in human, reduced and delayed onset of vocalization in infants is positively associated with subsequent diagnoses of neurodevelopmental disorders.

Disclosures: B. Zupan: None. **S.R.M. Dewil:** None. **L. Morse:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: NIH Grant R15SO88776

Title: Characterization of the behavioral phenotype and neuroinflammatory profile of the FMR1 KO mouse

Authors: *S. L. HODGES[1], S. O. NOLAN[2], C. REYNOLDS[2], G. D. SMITH[1], A. HOLLEY[2], T. JEFFERSON[2], J. HUEBSCHMAN[2], M. VOLQUARDSEN[2], A. PANDIAN[2], J. N. LUGO[3]; [1]Inst. of Biomed. Studies, [2]Psychology and Neurosci., [3]Psychology and Neuroscience, Inst. of Biomed. Studies, Baylor Univ., Waco, TX

Abstract: Fragile X syndrome (FXS) is a neurodevelopmental disorder characterized by a variety of behavioral, intellectual, and physiological abnormalities, caused by an expanded trinucleotide (CGG) repeat in the *FMR1* gene, leading to hypermethylation and subsequent functional silencing of expression of fragile x mental retardation protein (FMRP). In this current study, we examined social behavior, anxiety, locomotor activity, and repetitive behavior in adult male and female *FMR1* wild type (WT) and knockout (KO) mice on a FVB background strain. In comparison to WT mice, *FMR1* KO mice had significant increases in stereotypy time, rearing time, and total distance moved. The *FMR1* KO mice also displayed significant differences in anxiety, sociability, and learning. In addition to changes in the behavioral phenotype, recent studies have provided evidence of dysregulated cytokine and chemokine production in both

humans with FXS and mouse models indicating that an abnormal immune profile may also be associated with the syndrome. Increasing evidence has found elevated inflammatory markers and microglia dysfunction to be involved in the pathogenesis of autism, which is known to have a strong association with FXS. However, how this may relate to impaired behavioral outcomes is unknown. Following characterization of the behavioral phenotypes, we examined differences in neuroinflammatory markers, including IL-1β, TNF-α, and IL-10, as well as cell type expression in the hippocampus. With further characterization of the *FMR1* KO immune profile we hope to gain a better understanding of the potential pathogenic role of neuroinflammation in FXS and how this immune dysregulation may lead to the development of cognitive and behavioral deficits seen in the syndrome.

Disclosures: **S.L. Hodges:** None. **S.O. Nolan:** None. **C. Reynolds:** None. **G.D. Smith:** None. **A. Holley:** None. **T. Jefferson:** None. **J. Huebschman:** None. **M. Volquardsen:** None. **A. Pandian:** None. **J.N. Lugo:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: The effects of dietary supplementation with n-3 fatty acids on behavioral and neuroinflammatory phenotype of the Fmr1-knockout mouse.

Authors: *S. O. NOLAN[1], S. L. HODGES[2], G. D. SMITH[2], T. JEFFERSON[1], B. ESCOBAR[1], A. J. HOLLEY[1], J. N. LUGO[1];
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Abstract: Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by a genetic trinucleotide (CGG) overexpansion mutation in the fmr-1 gene coding for fragile x mental retardation protein (FMRP). This disorder is characterized by marked intellectual disability, as well as other autistic-like behavioral phenotypes. Supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFAs) has been proposed as an alternative treatment for a variety of neurodevelopmental disorders, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). In the present study, male FVB/129 fmr-1 knockout and fmr-1 wildtype littermates were assigned to one of three diet conditions following weaning on PD21: standard lab chow, EPA/DHA enriched chow, and a diet controlling for the

increase in fat associated with the EPA/DHA diet. Upon reaching postnatal day 90, animals were tested in a several behavioral assays, which included open field, elevated plus maze, social partition, nose poke assay, delay fear conditioning and pre-pulse inhibition. Preliminary results revealed that the increased dietary fatty acid composition significantly impacted sociability and fear learning in a genotype-dependent manner. No differences were noted in anxiety or repetitive behaviors. Previous research has indicated that dietary composition can affect the composition of the gut microbiome, which has downstream impacts on behavior and inflammation. Therefore, subsequent gut microflora, as well as western blotting and immunohistochemical analyses were performed to investigate molecular substrates associated with dietary and phenotypic changes.

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Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: PSPB-210

Title: Decrease of MMP-9 level in central amygdala rescues cognitive deficit in mouse model of fragile X syndrome

Authors: *A. PUSCIAN[1], S. LESKI[1], M. WINIARSKI[1], J. BOROWSKA[1], M. CHATURVEDI[1], J. SADOWSKA[1], H.-P. LIPP[2,3], E. KNAPSKA[1];
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Abstract: Loss-of-function mutations in the Fragile X mental retardation protein (FMRP) result in Fragile X syndrome (FXS), which is the most wide-spread single-gene cause of autism. FXS is a trinucleotide repeat disorder in which a CGG element located within the 5' untranslated region of *FMR1* gene expands and becomes hypermethylated. In case of the complete silencing of *FMR1* gene male patients have an average IQ of 40 and cope with severe cognitive impairments and excluding learning disabilities.
In our studies we exploited a mouse model of FXS (*Fmr1* knockouts) mimicking above-described phenotype in humans. As FMRP is a major local-translation suppressor its lack leads to overexpression of many synaptic plasticity proteins. Among those matrix metalloproteinase-9

(MMP-9) is a crucial player in reward-motivated learning, specifically its proper level in central amygdala (CA) is required for mice' ability to discriminate between highly-motivating reward and neutral stimuli.
Using fully automated behavioral testing (IntelliCage system), which enabled us to investigate characteristics that can be observed only in a continuous, long-lasting study, we showed that *Fmr1* knockouts are unable to efficiently perform such discrimination tasks. However, downregulation of heightened MMP-9 level in central amygdala was sufficient to fully rescue this impairment. This effect was obtained by a local injection of nanoparticles gradually releasing selective MMP-9 inhibitor (TIMP-1). It is noteworthy, that nanoparticles are able to cross brain-blood barrier and thus the implemented paradigm holds promise of obtaining clinically relevant solutions for the most severely disabled FXS patients.

Disclosures: **A. Puscian:** None. **S. Leski:** None. **M. Winiarski:** None. **J. Borowska:** None. **M. Chaturvedi:** None. **J. Sadowska:** None. **H. Lipp:** None. **E. Knapska:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: NIH Intramural Research Program

Autism Speaks Postdoctoral Fellowship #8679

Title: Chronic sleep restriction in Fmr1 knockout and WT mice has long term effects on behavior

Authors: *R. M. SARE, A. SONG, M. LEVINE, C. HILDRETH, A. MFON, C. SHEELER, C. BEEBE SMITH;
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Abstract: Sleep abnormalities are prevalent in patients with neurodevelopmental disorders, including those with Fragile X Syndrome (FXS). Furthermore, the severity of sleep abnormalities correlates with the severity of behavioral impairments. FXS is caused by an expanded CGG repeat on the X chromosome resulting in silencing of the *FMR1* gene and loss of the protein product FMRP. The physical and behavioral phenotypes of Fragile X are recapitulated in the *Fmr1* knockout (KO) mouse model. The behavioral phenotypes include hyperactivity, reduced anxiety, and social behavior abnormalities.Given the association between

sleep abnormalities and behavioral impairments, we sought to investigate the effects of chronic sleep restriction during a critical developmental period in KO mice on behavior in adulthood. We sleep-restricted both WT and *Fmr1* KO mice from P5-P42 for 3 hr per day by gentle handling. Following chronic sleep restriction, animals were allowed a period of four weeks of recovery sleep, after which we measured behavioral outcomes. We assayed activity and anxiety in the open field, social behavior, repetitive behavior, total sleep time, and sleep bout duration. In WT mice, sleep restriction resulted in hypoactivity, increased anxiety, reduced preference for social novelty, and shorter sleep bouts. In *Fmr1* KO mice, sleep restriction resulted in hypoactivity and longer sleep bouts; anxiety-like behavior and social behavior were unaffected by sleep restriction. Developmental sleep restriction resulted in phase-dependent effects on total sleep time suggesting a disruption in circadian rhythm; theses effects occurred in both genotypes. Repetitive behavior was unaffected by sleep restriction in either genotype. These behavioral changes highlight the importance of sleep during development and indicate that the response to sleep restriction is influenced by the absence of FMRP.

Disclosures: **R.M. Sare:** None. **A. Song:** None. **M. Levine:** None. **C. Hildreth:** None. **A. Mfon:** None. **C. Sheeler:** None. **C. Beebe Smith:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: FRAXA foundation

Azrieli Brain Canada

Title: Chronic administration of metformin restores behavioral and morphological abnormalities in the Fragile X Syndrome mouse model.

Authors: *I. GANTOIS[1], J. POPIC[1], A. KHOUTORSKY[1], E. FREEMANTLE[3], A. AGUILAR-VALLES[1], R. CAO[1], V. SHARMA[1], A. NAGPAL[1], K. GAMACHE[2], C. CHAPAT[1], T. POOTERS[4], K. NADER[2], J.-C. LACAILLE[3], C. G. GKOGKAS[4], N. SONENBERG[1]; [1]Dept. of Biochemistry, Rosalind and Morris Goodman Cancer Res. Ctr., [2]Dept. of Psychology, McGill Univ., Montreal, QC, Canada; [3]Dept. of Neurosciences, Univ. de Montreal, Montreal, QC, Canada; [4]Patrick Wild Ctr. and Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Fragile X syndrome (FXS), the leading single gene cause of autism and the most common form of hereditary mental retardation, is caused by the silencing of the *FMR1* gene and subsequent loss of fragile X mental retardation protein (FMRP). Loss of FMRP expression engenders enhanced activation of the mammalian target of rapamycin complex 1 (mTORC1) and extracellular signal-regulated kinase (ERK) signaling pathways in the brain of FXS patients and *Fmr1* knockout mice. The ERK pathway regulates mRNA translation largely via mitogen-activated protein kinase interacting kinases (MNK1/2)-dependent phosphorylation of eukaryotic initiation factor 4E (eIF4E), a cap binding protein that is critical for initiation of translation. Consistent with increased ERK activity, eIF4E phosphorylation is elevated in the brain of FXS patients and *Fmr1* knockout mice. The FXS mouse model recapitulates many core behavioral and morphological abnormalities of FXS patients such as social interaction deficits, repetitive behavior, dendritic spine dysmorphogenesis, and macroorchidism. In previous studies, downregulation of ERK and mTOR pathways rescued several pathologies in *Fmr1* knockout mice. Metformin, the most widely used anti-type 2 diabetic drug, represses the ERK and mTOR pathways, promotes lifespan, and protects against certain types of cancer and inflammation. In this study, we treated *Fmr1*$^{-/y}$ mice chronically with metformin. *Fmr1*$^{-/y}$ mice are impaired in the preference for social novelty part of the three-chamber social interaction task where they do not show a preference for the novel mouse. Meformin treatment corrected this impairment, where treated *Fmr1*$^{-/y}$ mice showed preference for the novel mouse similar as vehicle treated wild type mice. Increased grooming is a repetitive behavior observed in *Fmr1*$^{-/y}$ mice and metformin treatment decreased grooming comparable to wild type animals. Furthermore, metformin treatment rescued the excessive long-term depression (LTD), dendritic spine abnormalities and altered excitatory synaptic transmission in *Fmr1*$^{-/y}$ mice to the levels of vehicle treated wild type mice. Treatment with metformin also reduced the testicular weight in *Fmr1*$^{-/y}$ mice, but did not reach a weight of vehicle treated wild type mice. Using western blot, we showed that rescue of behavioral and morphological phenotypes in the *Fmr1*$^{-/y}$ mice after metformin treatment is due to selective normalization of ERK signaling pathway and consequently reduction of phospho-eIF4E, which is implicated in FXS. Together, metformin might be a novel therapeutic approach to restore specific behavioral and morphological aspects of FXS.

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Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: Medical Research Council

Department of Biotechnology, India

Wadhwani Foundation

The Shirley Foundation

The Patrick Wild Centre

RS MacDonald Trust

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Title: Lovastatin treatment early in life prevents development of cognitive deficits in a rat model of Fragile X Syndrome

Authors: *A. ASIMINAS[1,2], S. M. TILL[2,3], E. K. OSTERWEIL[3,2,4], M. F. BEAR[4], S. CHATTARJI[5,6], D. J. A. WYLLIE[2,3,5], P. C. KIND[2,3,5], E. R. WOOD[1,2];
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Abstract: Fragile X syndrome, is the leading inherited cause of intellectual disability and autism, affecting hundreds of thousands of people worldwide. While several pharmaceutical interventions have been shown to rescue pathophysiology associated with genetic deletion of FMRP in mice, little is known about whether cognitive deficits can be prevented. Furthermore, it is not known whether benefits of early drug treatment are permanent or require ongoing treatment. This study directly examines whether deficits in associative, “episodic-like” memory can be prevented in a rat model of FXS and whether any benefits are dependent on ongoing treatment. In this study we focused on the effects of lovastatin on cognitive deficits associated with genetic deletion of FMRP, since Osterweil et al (2013) showed that lovastatin can indirectly reduce the exacerbated ERK signalling in *Fmr1* KO mice as well as reverse cellular and behavioural deficits associated with the syndrome. LEH rats (n=24 WT, n=24 KO) were fed either a control chow or a “lovachow” (100mg/kg lovastatin-containing diet) between 29 and 64

days old, and tested in 4 spontaneous exploration tasks: object recognition (OR), object-context (OC), object-place (OP), and object-place-context (OPC) recognition. KO rats receiving control chow showed performance consistent with our previous results - i.e. KO rats were delayed in ability to discriminate OP associations, and unable to discriminate OPC associations throughout the experiment (Asiminas et al, SfN 2014. Poster 669.15). However, lovastatin-treated KO rats demonstrated a developmental profile of associative memory indistinguishable from that of WT animals, while WT animals receiving lovachow showed a normal developmental trajectory. We next tested whether the enhanced performance in lovastatin-treated KO rats required ongoing lovastatin application, or whether the beneficial effects of this treatment were maintained into adulthood after lovastatin application had ended. At P64, lovachow was replaced with standard laboratory chow and the animals were tested 1 and 3 months later. Surprisingly, lovastatin treated *Fmr1* KO animals maintained the ability to perform the OPC task even at 5 months of age, whereas *Fmr1* KO animals on control chow showed no improvement with age. Our results show that not only can lovastatin treatment prevent the emergence of cognitive deficits associated with Fragile X Syndrome but also that lovastatin (and perhaps pharmaceutical interventions more generally) may prevent the developmental deficits in neuronal circuit formation which can be maintained into adulthood.

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Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: Nancy Lurie Marks Foundation

Title: Modulation of behavioral inhibition in attention deficit hyperactivity disorder

Authors: ***J. REN**[1,3], A. PANAMENO[4], L. M. HIRSHBERG[2,5], L. M. OBERMAN[2,3]; [1]Psychiatry and Human Behaviors, Brown Univ., Riverside, RI; [2]Psychiatry and Human Behaviors, Brown Univ., Providence, RI; [3]Neuroplasticity and Autism Spectrum Disorder Program, [4]Bradley Hosptal, Riverside, RI; [5]The NeuroDevelopment Ctr., Providence, RI

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent child psychiatric disorder characterized by deficits in behavioral inhibition. One paradigm commonly used to evaluate behavioral inhibition is the stop signal task (SST). A cortical region implicated in this

task is the right inferior frontal gyrus (rIFG). Therefore, modulating the neural responses of the rIFG may lead to improvements in behavioral inhibition in individuals with ADHD.
The current study investigated this question using noninvasive brain stimulation. We applied anodal, cathodal, and sham stimulation over rIFG using transcranial direct current stimulation (tDCS) that provides a weak direct current to the scalp and examined the effects of stimulation on ADHD participants' performance on the SST as well as electroencephalography (EEG) and event related potentials (ERPs) related to behavioral inhibition. 15 adolescents aged 13-17 have been tested thus far. Participation consisted of 4 visits: a baseline and 3 stimulation visits (anodal, cathodal and sham). During each stimulation visit, participants received 15 minutes of tDCS over rIFG. Immediately after that, they completed the SST. During the SST, left or right arrows were presented on each trial and participants responded with a button press as quickly as possible. However, an auditory stop signal was presented in some trials instructing the participant to stop their responses. ERPs were recorded during the SST. A 5 minute resting EEG was recorded immediately prior to stimulation as well as following the SST.
Here we report interim analyses. Behavioral results showed a significant effect for stimulation on the participants ability to stop their responses ($F(3,12)=6.067$, $P=0.009$). Specifically, cathodal compared to sham stimulation lead to significant improvements (($F=4.819$, $P=0.045$)). Preliminary results for the resting EEG also suggested an important trend for reduced θ frequency and θ/β ratio at rIFG after stimulation for both anodal and cathodal conditions, suggesting that tDCS over rIFG may modulate neural correlates for behavioral inhibition. Data continues to be collected and further analyses will be conducted on the final dataset.
This is the first study to investigate the effects of a single session of tDCS over rIFG targeting behavioral inhibition in adolescents with ADHD. Current data indicate that tDCS to rIFG leads to transient improvements in behavioral inhibition and potentially the functioning of related neural circuitry. Such findings suggest that tDCS may be used as a novel clinical intervention for ADHD. Our findings also have significance in understanding the brain mechanism for inhibitory processes.

Disclosures: J. Ren: None. **A. Panameno:** None. **L.M. Hirshberg:** None. **L.M. Oberman:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Title: Children with DCD (development coordination disorder) have a normal rate of learning of Active Video Games both in a variable and repetitive learning protocol.

Authors: B. C. M. SMITS-ENGELSMAN[1], E. BONNEY[1,2], L. D. JELSMA[3], G. FERGUSON[1], *J. E. DUYSENS[4];
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Abstract: Introduction: Children with DCD (development coordination disorder) have difficulty in learning motor skills, yet under particular conditions they learn at the same rate as TDC (typically developing children (Smits-Engelsman et al. 2015). The latter was demonstrated using repetitive learning. Since there are indications that variable learning is superior to repetitive training (Schmidt 1975) the question arises whether variable practice leads to better learning and transfer than repetitive training. We examined if the rate of learning was different in these two training protocols. Moreover we tested if the transfer effect was different between protocols. Finally the question was asked if children with development coordination disorder (DCD) would benefit more from one of the protocols. Material and Methods: Some 111 children aged 6-10 (M 8.0 =, SD=1.0) with no active computer gaming experience were randomly divided in two groups. The first group (Variable, n=55) followed a training program with a variable protocol ("Variable", 10 different games) while the second group ("Same", n=56) followed a repetitive practice protocol (same game in every session). Half the participants in each group met the criteria for DCD. All subjects experienced 5 weeks of training two times per week, for 20 min. Both protocols aimed at learning dynamic balance and agility skills. Changes in outcome in a test game and in motor test scores were examined pre- and post-training. In addition their rate of learning was calculated over the 5 weeks of intervention. Results: ANOVA repeated measures indicated that, after the training, children submitted to the "Same" protocol outscored the ones in the "Variable" protocol on the test game (Protocol x Training). Importantly, there was no interaction effect between protocol (Same and Variable) and participant groups (TD and DCD). There was a significant transfer effect to the other balance tasks (both virtual and real life tasks) but transfer was not better for one of the protocols. Conclusions: By playing active computer games the motor performance,, especially balance, gets better, both in typically developing children and in children with poorer motor coordination. Our results confirm that there is transfer of training from video games to untrained tasks. The results do not confirm the generally held belief that DCD children are less able to learn. References: 1. Smits-Engelsman et al. PLoS One. 2015 2. Schmidt, Psychological Review, 1975

Disclosures: B.C.M. Smits-Engelsman: None. **E. Bonney:** None. **L.D. Jelsma:** None. **G. Ferguson:** None. **J.E. Duysens:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Topic: A.07. Developmental Disorders

Support: Seattle Children's Intercenter Funds

Title: Consequences of excessive sensory stimulation during development on addiction, impulsivity and attention

Authors: *S. RAVINDER, S. GADIWALLA, D. CHRISTAKIS, S. FERGUSON, J.-M. RAMIREZ;
Seattle Children's Res. Inst., Seattle, WA

Abstract: Early life experiences exert long-lasting effects on neural function, which can have a deep impact on behavior and vulnerability to developing neuropsychiatric illnesses such as Attention deficit hyperactivity disorder (ADHD). Most ADHD research has focused on understanding genetic causes, but there remains a large role for environmental factors in the etiology of this disease. We have developed a rodent model of "excessive sensory stimulation (ESS)" whereby mice are exposed to audio-visual stimuli for 6 h/day, from P10-P52 (Christakis et al., Sci Rep., 2012). When subsequently tested, ESS mice demonstrate numerous behavioral outcomes that are reminiscent of the clinical symptoms of ADHD (inattentiveness, impulsivity, hyperactivity). Compared to controls, ESS mice show poorer short-term memory, impaired learning and attention in memory tests; hyperactivity and increased risk-taking in multiple anxiety tests. ADHD is known to be highly comorbid with substance abuse. Here we investigated the relationship between ESS and vulnerability to drugs of abuse in adulthood. We found that ESS mice displayed locomotor hyperactivity that develops over time but showed blunted psychomotor sensitization to cocaine when compared to controls. Interestingly, ESS mice develop a stronger conditioned place preference for cocaine compared to controls, suggesting that they may have a higher addiction liability. To further characterize the behavioral impact of ESS, we are currently examining the effects of ESS in the 5-choice serial reaction time test and the delay-discounting test, both of which are well-established models for testing impulsivity and attention. Our initial data from the delay discounting test suggests that ESS mice make more impulsive choices than controls. At the cellular level, our experiments show that ESS leads to an increase in miniature excitatory postsynaptic currents in the nucleus accumbens, amygdala and medial prefrontal cortex (mPFC). We are currently pursuing experiments to further characterize these cellular effects. Many studies have shown that changes in the frontal cortex may underlie impulsivity and attention in animal models and ADHD symptoms in humans. In future experiments, we plan to modulate *in-vivo* activity in the mPFC of ESS and control mice to

explore the role of this brain region in ESS induced changes in impulsivity and attention. Our study has important implications in today's increasingly complex technological age where children face a tremendous amount of sensory stimulation that could interact with genetic predispositions to produce detrimental effects on behavior and brain function.

Disclosures: S. Ravinder: None. **S. Gadiwalla:** None. **D. Christakis:** None. **S. Ferguson:** None. **J. Ramirez:** None.

Poster

392. Behavior in Fragile X Syndrome and Other Neurodevelopmental Diseases

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 392.11/C28

Topic: A.07. Developmental Disorders

Support: Wallace Research Foundation

James Gates Family Foundation

Kawaja-Holcombe Family Foundation

Title: Attention training in children with sensory processing dysfunction.

Authors: *A. AITKEN, J. A. ANGUERA, A. D. ANTOVICH, C. E. ROLLE, S. S. DESAI, E. J. MARCO;
UCSF, San Francisco, CA

Abstract: Background:
Children with Sensory Processing Dysfunction (SPD) have profound behavioral issues stemming from their atypical behavioral responses to incoming sensory stimuli and, frequently, attention-based deficiencies. It has been estimated that 40% of children with SPD will meet diagnostic criteria for Attention Deficit and Hyperactivity Disorder (ADHD). However, there is uncertainty regarding the most effective treatment option at an individual level, especially when considering non-pharmaceutical approaches.
Objectives:
In the current study, we present behavioral and neuroimaging work aimed to determine if training with an interactive digital game-like tool (EVO) would enhance attention in children with SPD relative to age-matched controls.
Methods:
We recruited 19 typically developing children (TDC), 17 children with SPD and inattentive traits (SPD_{+IA}) and 13 children with SPD without inattentive traits (SPD_{-IA}) through the UCSF SNAP

Center. All children played EVO over a one-month period. Pre and post training, we assessed response time (RT) and response time variability (RTV) in the Test of Variables of Attention (TOVA), a Go/NoGo assessment, EVO assessment, a neurophysiological measure of cognitive control (Midline Frontal Theta using EEG), and a parent report measure of inattention (Vanderbilt).

Results:

Using an linear mixed model analysis, we found a significant main effect by session for TOVA RT ($F(1,56)= 13.6$, $p= .001$) and RTV ($F(1,56)= 6.0$, $p= .02$), Go/NoGo RT ($F(1,54)= 26.5$, $p<.001$,), and EVO RT ($F(1,52)= 103.1$, $p \leq .001$) and RTV ($F(1,52)= 45.3$, $p\leq .001$). Midline frontal theta (MFT) power during Go/NoGo play showed a significant group x visit interaction ($F(2,47)= 5.9$, $p= .007$), with post-hoc tests revealing that only the SPD_{+IA} group showed enhanced MFT power following training ($p\leq .001$). The Vanderbilt parent report also showed a significant group x session interaction ($F(2,61)= 9.8$, $p\leq .001$), with post-hoc tests revealing that only the SPD_{+IA} group showed reduced inattention ($p\leq .001$), with these improvements persisting for 9 months. Critically, we observed a significant relationship between increased MFT power and decreased parent reported inattention via the Vanderbilt ($r=.41$, $p=.017$).

Conclusions:

These findings support the benefit of a specific targeted attention-based intervention for children with SPD and symptoms of ADHD, with evidence of brain plasticity and impact on real world function. These outcomes also highlight the importance of having a detailed cognitive assessment of attention in children with neurodevelopmental conditions to properly personalize subsequent treatment approaches.

Disclosures: **A. Aitken:** None. **J.A. Anguera:** None. **A.D. Antovich:** None. **C.E. Rolle:** None. **S.S. Desai:** None. **E.J. Marco:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.01/C29

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant MH081187

Title: C-terminal RELN deletion disrupts an interaction with VLDLR causing abnormal cerebral cortex and hippocampus development

Authors: *S. HA[1,3], P. P. TRIPATHI[2], R. F. HEVNER[2,3], D. R. BEIER[1,3];
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Abstract: We discovered a hypomorphic *reelin* (*Reln*) mutant with abnormal cortical lamination and no cerebellar hypoplasia. This mutant, $Reln^{CTRdel}$, carries a splice-site mutation that truncates the C-terminal region (CTR) domain and displays remarkably distinct phenotypes from *reeler*. The mutant does not have an inverted cortex, but cortical neurons overmigrate and invade the marginal zone, which is very similar to a phenotype seen in the cerebral cortex of $Vldlr^{null}$ mice. Moreover, the dentate gyrus shows a novel phenotype; the infrapyramidal blade is absent, while the suprapyramidal blade is present and laminated. Genetic epistasis analysis showed that $Reln^{CTRdel}/Apoer2^{null}$ double homozygotes have phenotypes akin to *reeler*, while $Reln^{CTRdel}/Vldlr^{null}$ mice do not. Given that the receptor double-knockout mice resemble *reeler,* we infer that $Reln^{CTRdel}/Apoer2^{null}$ double homozygotes have both receptor pathways disrupted. Therefore, these results suggest that CTR-truncation disrupts an interaction with VLDLR, while the APOER2 signaling pathway remains active, which accounts for the hypomorphic phenotype in $Reln^{CTRdel}$ mice.

Disclosures: S. Ha: None. **P.P. Tripathi:** None. **R.F. Hevner:** None. **D.R. Beier:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.02/C30

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI Grant Number 26670093

Title: A possible novel mode of action for Dab1 in modulation of neuronal migration of neocortical neurons

Authors: *S. KIKKAWA, T. NAMIKAWA, T. TERASHIMA;
Kobe Univ. Grad. Sch. of Med., Kobe-Shi, Japan

Abstract: Neurons in the developing mammalian neocortex form a laminar structure in an ﾒinside-outﾓ manner. This layered structure is established by coordinated neuronal migration regulated by several signaling cascades, including the Reelin-Disabled-1 (Dab1) pathway. Dab1 comprises a N-terminal (Nt) region, which contains the phosphotyrosine-binding (PTB) domain followed by a cluster of five tyrosines, and a C-terminal (Ct) region with unknown functions. We

have previously investigated the effects of various truncated forms of Dab1 on the morphology of transfected cells and postulated a possible intramolecular regulatory mechanism between the Nt and Ct domains within Dab1. To explore this interaction *in vivo*, we expressed various Nt and Ct fragments in migrating neocortical neurons using an *in utero* electroporation technique and analyzed radial migration of the transfected neurons. When transfected with the Dab1 Nt fragment along with a GFP tracer plasmid at E14.5, most neurons, which normally comprise layer II/III, ceased migration and accumulated ectopically in the intermediate zone. The site of accumulation and the morphology of accumulating cells suggest that these cells failed to switch from multipolar migration to bipolar locomotion. This premature migration arrest occurred irrelevantly to phosphorylation in the tyrosine cluster within the Dab1 Nt fragment and required neither endogenous Reelin nor Dab1. When the Nt fragment was cotransfected with its complementary Ct fragment, the migration arrest was partially rescued to the extent comparable to the case of full-length Dab1 transfection. Intriguingly, overexpression of either constitutively active Rap1 or a Rap1 guanine nucleotide exchange factor, RapGEF2, also rescued the Nt-fragment-mediated migration arrest. Taken together, these results suggest that the Dab1 Ct domain interacts with the Nt counterpart so that it modulates the activity of the Nt domain, and also that Dab1 may act in the RapGEF2-Rap1 pathway upon multipolar-bipolar transition of migrating neocortical neurons.

Disclosures: **S. Kikkawa:** None. **T. Namikawa:** None. **T. Terashima:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.03/C31

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSC Grant 101-2320-B-010-064

MOST Grant 104-2320-B-010-022-MY3

MOE

Title: Cdk12 regulates neurogenesis and late-born neuronal migration in the developing cerebral cortex

Authors: ***M.-J. FANN**, H.-R. CHEN, H.-C. JUAN, Y.-H. WONG, J.-W. TSAI;
Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The DNA damage response (DDR) pathways are critical to ensure that replication stress and other types of DNA lesion do not perturb production of neural cells during development. Neural progenitor cells are particularly sensitive to DNA damage and their relative susceptibility may vary depending on the stage of development. Defective DDR pathways are often associated with neural developmental abnormality. Cdk12 maintains genomic stability by regulating expression of DDR genes. To define its role during neural development *in vivo*, we conditionally targeted *Cdk12* using a Nestin-Cre mouse line. Nestin-Cdk12-cKO mice do not survive beyond postnatal day 0 (P0) and exhibit microcephaly. Results from cresyl violet-stained brain sections of Nestin-Cdk12-cKO at P0 show reduced thickness of cortical plate layers in cerebral cortex, and aberrant anterior commissure and corpus callosum. We show that neural progenitor cells of mutant mice accumulate at G2 and M phase, and have lower expression of DDR genes, more DNA double-strand breaks and increased apoptosis. Furthermore, results from birthdating experiments show that Cdk12 involves not only in neurogenesis of neural progenitor cells, but also in neuronal migration of late-born neurons in the developing cerebral cortex. Although 6-cortical layer organization is preserved, misaligning of layers IV–II neurons marked with CUX1 expression is observed. Results from DiI tracing also show a loss of the corpus callosum that is composed of axons of callosal projection neurons located in layer III/II. To further investigate effects of Cdk12 on neuronal migration without its confounding effect on neurogenesis, we investigated the $Cdk12^{fx/fx}$ mice that were electroporated *in utero* with Cre-expression plasmid between embryonic day 14 to 16. Migrating mutant neurons are stagnant within the intermediate zone and fail to adopt a bipolar morphology. As Cdk12 is required for Cdk5 expression, we demonstrate that overexpression of Cdk5 brings about a partial restoration of the neurons reaching layers IV-II in the mutant mice. Thus, Cdk12 is crucial for the repair of DNA damage during the proliferation of neural progenitor cells and is also central to the proper migration of late-arising neurons.

Disclosures: **M. Fann:** None. **H. Chen:** None. **H. Juan:** None. **Y. Wong:** None. **J. Tsai:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.04/C32

Topic: A.05. Axon and Dendrite Development

Support: CURE pediatric epilepsy award

Title: Loss-of-function of the epileptic encephalopathy-associated TRIO gene impairs the morphological development of cortical GABAergic interneurons.

Authors: *F. CHARRON-LIGEZ[1,2], J.-D. LAROUCHE[1,3], F. HANSSON[1], M. LACHANCE[1], J. FALARDEAU[1,4], E. ROSSIGNOL[1,4,5];
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Abstract: Epileptic encephalopathies (EEs) are neurodevelopmental diseases characterized by early-onset epilepsy with cognitive deficits. The etiology of these disorders remains uncertain in a large proportion of patients. However, recent data suggest that defects in the development of inhibitory interneurons (INs) may perturb the connectivity and aberrantly enhance the excitability of cortical circuits, underlying a subset of genetically determined seizure disorders. Mutations in the *TRIO* gene have been recently identified using whole-exome sequencing in patients with EE or isolated intellectual deficiency by our lab and others. *Trio* encodes a guanine nucleotide exchange factor (GEF) that interacts with multiple Rho GTPases, such as Rac1, RhoG and RhoA, implicated in fundamental aspects of neuronal development, including cell cycle regulation, neurite outgrowth, nucleokinesis and caudal neurite retraction. *Trio* has been shown to regulate the axonal guidance, dendritic development and lamination of hippocampal pyramidal cells. However, the roles of *Trio* in the development INs are unknown. Considering the proposed involvement of *Trio* in neuronal development, and given the critical role of INs in preventing seizure disorders, we hypothesized that *Trio* might be an important regulator of the development and connectivity of cortical INs. To investigate this hypothesis, we performed a targeted repression of the *Trio* gene by *ex vivo* electroporation of a *Dlx5/6::shRNA-tdTomato* plasmid in organotypic cultures of e13.5 mice embryonic cortex. Using time-lapse confocal imaging, we evaluated the morphology and dynamics of migrating INs. We also investigated the migration dynamics of genetically modified INs from MGE-explants plated on a cortical feeder layer. Finally, we genetically repressed *Trio* in post-natal cortical parvalbumin-positive (PV) INs by biolystic transfection of a 10kb-GAD67::shRNA in postanatal cortical organotypic cultures. We assessed the morphological development of PV-INs and the extent of their innervation of cortical pyramidal cells. Our preliminary results show that the repression of *Trio* in migrating cortical INs significantly alters their morphology and impairs their migration. This data suggests that *TRIO*-associated EE might be, in part, due to a defect in the early development of cortical INs.

Disclosures: F. Charron-Ligez: None. **J. Larouche:** None. **F. Hansson:** None. **M. Lachance:** None. **J. Falardeau:** None. **E. Rossignol:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.05/C33

Topic: A.01. Neurogenesis and Gliogenesis

Support: Chateaubriand Fellowship

Jim and Betty Ann Rodgers Chair Fund

Title: Dopamine D1 receptor activation and neuronal migration

Authors: ***M. M. MARTIN**[1,2], D. M. MCCARTHY[2], C. METIN[3], P. G. BHIDE[2];
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Abstract: Neurotransmitters play unique growth-promoting roles in the developing nervous system, which are distinct from their canonical role at the synapse in the mature nervous system. We have shown that the neurotransmitter dopamine (DA) and its receptors not only appear in the fetal brain during early development but also play critical roles in developmental phenomena such as cell proliferation, neuronal migration, differentiation and circuit formation. Perturbation of these developmental events is believed to be associated with a number of neurological and psychiatric disorders with developmental onset. Many of these developmental disorders are also associated with impairment of inhibitory GABA circuits. GABA neurons originate in the dopamine-rich medial ganglionic eminence (MGE) of the embryonic basal forebrain and migrate to regions of the dorsal forebrain. These migrating GABA neurons express dopamine receptors. Therefore, we hypothesized that dopamine receptor activation influences migration of the GABA neurons. Using live cell imaging techniques, we examined the migratory behavior of these GABA neurons following application of the DA type 1 receptor (D1R) agonist SKF 81297. We used a co-culture system in which MGE explants were plated on dissociated cortical neurons; both obtained from embryonic day 13 mice. Following 24 hours in culture, the D1R agonist (1 μM) was applied and live cell imaging was performed over a 7 hr period. We found the migration velocity of treated GABA neurons to be significantly decreased by 28% compared to those treated with a vehicle control. Migrating GABA neurons typically display a saltatory behavior characterized by the alternation between a nuclear jump-like movement and a nuclear resting phase. We found that D1R agonist application significantly increased the average time that GABA neurons spent in the resting phase by 35%. Moreover, D1R agonist application significantly decreased the frequency of medium jumps (characterized by an instantaneous migration velocity between 1.2 and 2.4 μm/min) by 30% and the frequency of large jumps (characterized by an instantaneous migration velocity greater than 2.4 μm/min) by 36%. Collectively, these results demonstrate that D1R activation can alter the migratory behavior of GABA neurons during early brain development suggesting a role for impaired D1R activity in the etiology of developmental disorders associated with dopamine imbalance.

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Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.06/C34

Topic: A.01. Neurogenesis and Gliogenesis

Support: HI 678/8-1

Title: Polysialic acid synthesis by ST8SIA2 is essential for cortical interneuron development

Authors: *U. E. DIEDERICHS[1], C. ROSSDAM[1], T. KRÖCHER[1], I. RÖCKLE[1], N. KESSARIS[2], Y. YANAGAWA[3], B. WEINHOLD[1], H. HILDEBRANDT[1];
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Abstract: The neural cell adhesion molecule NCAM and its modification with polysialic acid (polySia, PSA-NCAM) are major determinants of brain development. Polysialylation of NCAM is implemented by the polysialyltransferases (polySTs) ST8SIA2 and ST8SIA4. Dysregulation of polySia-NCAM and variation in *ST8SIA2* have been implicated in psychiatric disorders such as schizophrenia. Previously, we described reduced interneuron densities in the prefrontal cortex of ST8SIA2- or ST8SIA4-negative mice and demonstrated that acute enzymatic removal of polySia in organotypic slice cultures causes impaired entry of interneurons into the embryonic cortex as well as slower migration within the cortical environment (Kröcher et al. 2014, Development 141:3022). However, the spatiotemporal impact of the two polySTs on the migration process remained unresolved. Here we characterize altered interneuron migration by live cell imaging of ST8SIA2-deficient embryos. To distinguish between a cell-autonomous effect of polySia produced by ST8SIA2 in migratory interneurons and its role in the cortical environment, the migration of GFP-labelled interneurons from the medial ganglionic eminence (MGE) into the cortex was studied in MGE- and cortex-explants obtained from wildtype or ST8SIA2-deficient embryos that were co-cultured in different combinations. A potential link between cell-autonomously altered polySia and reduced cortical interneuron densities was assessed by analyzing mice with a conditional knockout of *St8sia2* either in MGE-derived interneurons (*Lhx6-Cre*) or in the cortex (*Emx1-Cre*). The results point towards a differential impact of polySia produced by ST8SIA2 in migratory interneurons and in their cortical environment on the migration process as well as on the establishment of interneuron densities in the mouse cortex.

Disclosures: U.E. Diederichs: None. **C. Rossdam:** None. **T. Kröcher:** None. **I. Röckle:** None. **N. Kessaris:** None. **Y. Yanagawa:** None. **B. Weinhold:** None. **H. Hildebrandt:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF-2012M3A9C6049933

NRF-2011-0019210

NRF-2015M3C7A1028790

NRF-2013R1A1A3011896

HI14C3347

Title: Dynamin-related protein 1 controls the migration and neuronal differentiation of subventricular zone-derived neural progenitor cells

Authors: *H. KIM[1], M. SHAKER[1], B. CHO[2], H. CHO[1], H. KIM[1], J. KIM[1], W. SUN[1]; [1]Korea Univ., Seoul, Korea, Republic of; [2]DGIST, Daegu, Korea, Republic of

Abstract: Mitochondria mediate many cellular functions required for cell survival and maintenance, as they play a prominent role in energy production and calcium homeostasis. The organelles are scattered throughout the cytoplasm, but their distribution can be altered in response to local energy demands, such as cell division and neuronal maturation. Mitochondrial distribution is closely associated with mitochondrial fission, and blocking dynamin-related protein 1 (Drp1) activity often results in mitochondrial elongation and clustering. In this study, we observed that mitochondria were preferentially localized at the leading process of migratory adult neural stem cells (aNSCs), whereas neuronal differentiating cells transiently exhibited perinuclear condensation of mitochondria. Inhibiting Drp1 activity altered the morphology of migrating cell from elongated to round, while the polarized mitochondrial distribution was maintained. With these changes, aNSCs failed to migrate and differentiate. Because blocking Drp1 activity also impaired the mitochondrial membrane potential, we tested whether supplementing with L-carnitine, a compound that restores mitochondrial membrane potential and ATP synthesis, could rescue the defects induced by Drp1 inhibition. Interestingly, L-carnitine fully restored the impaired cell morphology, migration and differentiation in aNSCs. These results suggest that Drp1 is important for regulating mitochondria to produce ATP for proper migration and differentiation, and supplementing with ATP can restore the defects induced by Drp1 suppression.

Disclosures: **H. Kim:** None. **M. Shaker:** None. **B. Cho:** None. **H. Cho:** None. **H. Kim:** None. **J. Kim:** None. **W. Sun:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.08/D2

Topic: A.01. Neurogenesis and Gliogenesis

Support: EPSRC studentship

Title: Insulin growth factor 1 control of subventricular zone progenitor migration

Authors: ***M. DUCKER**[1], B. HASSAN[2], F. SZELE[1];
[1]Dept. of Physiol. and Genet., [2]Sir William Dunn Sch. of Pathology, Univ. of Oxford, Oxford, United Kingdom

Abstract: The subventricular zone (SVZ) of the lateral ventricles is one of the two major neurogenic niches in the adult mammalian brain. SVZ neural stem cells proliferate and generate thousands of highly motile neuroblasts daily that migrate to the olfactory bulb or, under pathological conditions, to the site of injury. Evidence from the literature supports the hypothesis that the Insulin-like Growth Factor (IGF) system orchestrates both the proliferation and migration of SVZ progenitors. However, it is unknown how the balance between proliferation and migration is achieved.
We employed in vitro explant assays and two photon time lapse microscopy of neuroblasts migrating in live brain slices to study the role of IGF signalling in the SVZ. This is combined with fluorescence resonance energy transfer (FRET) biosensors to correlate IGF stimulation of key kinase pathways with the dynamics of proliferation and migration to examine the effects of IGF signalling on SVZ migration.
Stimulation with recombinant IGF1 increased migration of neuroblasts from SVZ explants. This effect was blocked by small molecule inhibitors of the IGF1R and the PI3K and MAPK pathways. Co-treatment with the Insulin-like growth factor binding protein 5 (IGFBP5) potentiated the promigratory effect of IGF1, while treatment with IGF2 failed to elicit significant increases in migration. Interestingly in these assays epidermal growth factor (EGF) induced migration of cells with non-neuroblast morphology. We are currently working to fully characterise the expression of the different components of the IGF system on the different cell types in the SVZ. Targeted manipulation of the IGF system may offer therapeutic potential for directed neuroblast migration in neuroregeneration.

Disclosures: **M. Ducker:** None. **B. Hassan:** None. **F. Szele:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

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Program#/Poster#: 393.09/D3

Topic: A.01. Neurogenesis and Gliogenesis

Support: Suomen Akatemia (nr 259799)

Sigrid Juselius Foundation

ANR-13-BSV4-0012-01

Suomen Akatemia (nr 266820)

DPBM

Title: *In vivo* two-photon imaging of cell migration in embryos reveals a differential effect of ketamine application

Authors: ***M. YURYEV**[1], L. ANDRIICHUK[1], V. JOKINEN[2], C. RIVERA[1,3,4];
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Abstract: Correct neuronal migration is an essential part of the process of cortical development. Alterations in the mechanism regulating speed of migration may have profound consequences in the incorrect structure of cortical networks. Individual cellular populations mature at various rates and might respond to different extracellular cues. The varying effects might stem partly from differential expression of glutamatergic and GABAergic receptors. However, the functionality of these receptors in different cellular populations remains unclear. To date, the physiological behaviour of migrating neurons has been mainly studied in *ex vivo* models such as neuronal cultures and brain slices. These reduced systems do not necessarily reflect the complexity of molecular and electrical signalling of the brain *in vivo*. In the present work we took advantage of a recently developed method of *in vivo* two-photon imaging of live mouse embryos connected to the mother at gestational day E14. We used Sox2-GFP reporter mouse to track the migration of undifferentiated cells. Then we exploited the ability of Fluo4-AM dye to selectively label differentiated neurons after *in vivo* intraventricular dye loading. With this approach we study the differences in migration of this cell population in comparison to Sox2-positive cells. We injected ketamine intraventricularly in order to block NMDA receptors. This

treatment induced reduction in the migration speed in both populations. However, in contrast to Sox2-positive cells, ketamine application had significantly stronger effects in differentiated neurons. The results imply the involvement of NMDA receptors selective regulation of migrating neurons *in vivo*. In addition, this method could be used to estimate the effects of maternal exposure to chemicals on acute cellular migration and and thus serve as a drug screening platform.

Disclosures: **M. Yuryev:** None. **L. Andriichuk:** None. **V. Jokinen:** None. **C. Rivera:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.10/D4

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 5R01HL110791

Brown University Seed Grant

Title: Understanding neurovascular endothelial cell migration using *In vitro* three dimensional models

Authors: M. BOUTIN[1], J. SEVETSON[2], L. KRAMER[3], *D. HOFFMAN-KIM[4];
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Abstract: The neurovascular unit is a complex environment composed of diverse neural cell types and basement membrane proteins, which coordinate to deliver oxygen and nutrients to the brain, and to protect the brain from toxins and metabolic fluctuations in the blood stream. Pathological changes to neural vasculature occur in many disease states, such as cancer, stroke, and neurodegeneration. Understanding the cellular dynamics behind pathological vascular changes is an imperative step towards the development of effective therapeutics. To this end, our lab has characterized the formation and dynamics of *in vitro* neurovascular capillary-like networks. *The goal of the current study was to characterize the response of capillary-like networks to the introduction of different cell types, to mimic potential in vivo disease states.* We previously described a primary cortical spheroid model that enables the *in vitro* study of *in vivo*-relevant characteristics, such as cell density, cell composition complexity, neuronal electrophysiology, tissue stiffness, and dimensionality. Importantly, cortical endothelial cells spontaneously assemble into capillary-like network structures within cortical spheroids. In the current study, a coculture method was used to study the fusion of a cortical spheroid and a

spheroid composed of a different cell type. The fibroblast cell line NIH3T3 and the endothelial polyoma cell line bEnd.3 were selected to create candidate coculture spheroids. After coculture of cortical spheroids and NIH3T3 spheroids, the two discrete spheroids fused to form one microtissue, and capillary-like networks were observed within NIH3T3 regions of fused microtissues. After coculture of cortical spheroids and bEnd.3 spheroids, capillary-like structures were observed at the interface between the fused spheroids. These results demonstrate the complex differential responses of cortical capillary-like networks to introduced cell types. Future work aims to investigate the interaction of capillary-like networks with different introduced cell types and to identify the driving factors of endothelial cell migration, with the goal of informing vascular changes in disease states.

Disclosures: **M. Boutin:** None. **J. Sevetson:** None. **L. Kramer:** None. **D. Hoffman-Kim:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

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Program#/Poster#: 393.11/D5

Topic: A.01. Neurogenesis and Gliogenesis

Support: 2R15NS060099-03

1SC3GM096904-01

Title: The effect of migration in Schwann cell line using NRG1, NGF, and GDNF as guidance signals

Authors: ***M. E. DE BELLARD**[1], B. ORTEGA[1], T. DUONG[1], E. KLEIN[1], J. KOWALEWSKI[1], A. MAYORAL[2];
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Abstract: Derived from the neural crest, Schwann cells are the nonmotile, myelinating glia of the peripheral nervous system. Although the transitional stages of the embryonic Schwann cell have been characterized, the guidance signals have yet to be determined. Since neuroregulin-1 (NRG1) is essential for survival and generation of Schwann cell development, we decided to include neurotrophins also known to be present in the developing embryo and important for the survival of glia in the peripheral nervous system (PNS). More specifically, we want to know if NRG1, nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) are chemoattractants and/or chemokinetic molecules for Schwann cells. We implemented a

chemoattraction assay with live imaging in addition to chemokinetic assays to measure motility. Our findings suggest NRG1 and NGF are strong chemoattractants for Schwann cells while NRG1 and GDNF are chemokinetic molecules for their motility.

Disclosures: **M.E. De Bellard:** None. **B. Ortega:** None. **T. Duong:** None. **E. Klein:** None. **J. Kowalewski:** None. **A. Mayoral:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

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Program#/Poster#: 393.12/DP01 (Dynamic Poster)

Topic: A.01. Neurogenesis and Gliogenesis

Support: Ministry of Education, Science, Technology, Sports and Culture of Japan (25123727, 25430046)

Title: Erratic migration: a unique migratory behavior of astrocyte progenitors

Authors: ***H. TABATA**[1,2], M. SASAKI[2], Y. INAGUMA[1], H. ITO[1], H. TAKEBAYASHI[3], M. EMA[4], K. IKENAKA[5], K.-I. NAGATA[1], K. NAKAJIMA[2];
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Abstract: During cerebral cortical development, neurons and glia are produced directly or indirectly from neural stem cells in the ventricular zone and migrate to their final destinations. Although the migratory process and its molecular mechanisms of cortical neurons are well described, those of glial progenitors are largely unknown. During our observations of the cells migrating from the cortical ventricular zone, we have noticed that some cells moved in a very unique manner that had not been previously described: these cells moved very rapidly and almost randomly within the intermediate zone and the cortical plate and frequently divided. We named this migration “erratic migration”. The lineage analyses of them both in vitro and in vivo revealed that they were astrocyte progenitors destined for cortical gray matter. Interestingly, these cells frequently migrated along blood vessels, which are running radially in the cortical plate during the embryonic and perinatal stages, and reached superficial layers of the cortical plate. The molecular mechanism of the blood vessel-guided migration will be discussed.

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Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.13/D6

Topic: A.01. Neurogenesis and Gliogenesis

Title: EphA4 signaling controls neuroblast migration and astrocyte organization in the rostral migratory stream

Authors: ***J. C. CONOVER**[1], K. L. TODD[2], K. L. BAKER[2], M. EASTMAN[2], F. KOLLING, 4th[2], C. E. NELSON[3];
[1]Dept Physiol & Neurobiol, Univ. Connecticut, Storrs Manfld, CT; [2]Physiol. and Neurobio., [3]Mol. and Cell. Biol., Univ. of Connecticut, Storrs, CT

Abstract: Newly generated neuroblasts from the subventricular zone (SVZ) migrate from the lateral ventricle through the anterior forebrain to their ultimate site of differentiation in the olfactory bulb. The migration pathway through the forebrain, the rostral migratory stream (RMS), consists of densely packed, tangentially oriented neuroblasts transiting through a meshwork of astrocytes. To support efficient and controlled migration of neuroblasts, the RMS needs to be tightly regulated, spatially restricted, and yet permissive. What regulates the dynamic interaction between migratory neuroblasts and the glial meshwork that makes up the RMS? We found that EphA4 tyrosine kinase receptor signaling is required to confine neuroblasts within the RMS and to organize the astroglial meshwork. Single cell analysis revealed that EphA4 and its ephrin partners are expressed in a complex mosaic pattern by subpopulations of neuroblasts and astrocytes within the RMS, implicating EphA4 signaling in both neuroblast-neuroblast and neuroblast-astrocyte interactions as crucial for regulating the dynamic interplay between neuroblast migration and controlled boundary maintenance supported by RMS astrocytes. In sum, our data supports a novel molecular mechanism involving EphA4 signaling that is necessary for controlling neuroblast migration in the RMS.

Disclosures: **J.C. Conover:** None. **K.L. Todd:** None. **K.L. Baker:** None. **M. Eastman:** None. **F. Kolling:** None. **C.E. Nelson:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.14/D7

Topic: A.01. Neurogenesis and Gliogenesis

Support: Intramural Research Program of the National Institutes of Health, National Institute of Neurological Disorders and Stroke Grant ZIA NS002824-26

Title: Cytoskeletal dynamics during neuronal migration: Role of actin binding protein drebrin in GnRH neuronal movement

Authors: *Y. SHAN, S. WRAY;
NINDS, NIH, Bethesda, MD

Abstract: Gonadotropin-releasing hormone (GnRH) neurons are essential for reproductive maturation and function. In vertebrates, GnRH neurons migrate from the nasal pit into the brain during embryonic development. Numerous guidance molecules are involved in this process to ensure GnRH neurons reach their final destination, since disruption of migration can result in infertility. GnRH cell movement is regulated through cytoskeletal modification in response to these guidance cues. These cytoskeletal changes include rapid assembly/disassembly of actin filaments and microtubules, and extension of the leading process and growth cones. However, the exact mechanisms by which guidance cues 'talk' to the cytoskeleton of GnRH neurons remain unclear. Previous data in our lab indicated that 1) stromal cell-derived factor 1 (SDF-1) accelerates GnRH neuronal migration rate and 2) 'capturing' microtubule + ends to cortical actin in migrating GnRH neurons occurs via calcium dependent mechanisms. It is known that SDF-1 binds to CXCR4, a g-protein coupled receptor that can increase release of calcium from internal stores. Large numbers of actin/microtubule binding proteins regulate cytoskeletal dynamics during neuronal migration, including developmentally regulated brain protein (drebrin), which is thought to stabilize actin filaments. Drebrin has been shown to interact with microtubule end-binding proteins through cdk signaling pathways to regulate cytoskeletal dynamics and has been proposed to regulate Ca^{2+} influx through actin organizing. Thus drebrin is a candidate actin/microtubule binding protein that may link receptor signaling to cytoskeletal changes during neuronal migration. In the current study, we identified that drebrin is expressed along the actin cortex and growth cone of migrating GnRH neurons, but not in the olfactory/vomeronasal axons along which GnRH cells migrate. In migrating GnRH neurons, drebrin was also colocalized with the microtubule-associated protein double cortin (DCX), consistent with a role for drebrin guiding the microtubule bundles into the actin cortex. Functional assays have been performed using embryonic mouse nasal explants that maintain large numbers of primary GnRH neurons migrating in association with outgrowing olfactory axons. Inhibition of drebrin using the calcium

release activated channel (CRAC) inhibitor BTP2 decreased GnRH neuronal migration rate, consistent with drebrin being involved in the mechanism of cytoskeletal modification. Further work will focus on the interaction of the SDF-1 receptor, CXCR4, and drebrin during GnRH neuronal migration.

Disclosures: **Y. Shan:** None. **S. Wray:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.15/D8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant ZIA NS002824-26

Title: Does GPR56 play a role in the developing GnRH/Olfactory system?

Authors: ***F. VILSON**[1], Y. SHAN[1], X. PIAO[2], S. WRAY[1];
[1]NIH, Bethesda, MD; [2]Div. of Newborn Med., Boston's Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: The Gonadotropin Releasing Hormone-1 neuron (GnRH-1) system controls reproductive maturation and function via the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the pituitary gland. During embryonic development, GnRH neurons migrate into the forebrain from the nasal placode. The factors/signals that guide these cells into the forebrain are not fully understood. Microchip data obtained from single GnRH neurons maintained in nasal explants indicated a high level of transcripts for GPR56. GPR56 is a member of the adhesion class of the G protein coupled receptor superfamily. Previous reports indicate that mice with mutations in the GPR56 gene exhibit a lack of structural integrity of the cerebral cortex and over-migration of cortical neurons, moving beyond the pial basement layer. The present study examines whether GPR56 plays a role in GnRH neuronal migration. Single cell RT-PCR confirmed expression of GPR56 transcript in GnRH neurons during migration and immunocytochemistry showed a subpopulation of GnRH cells express the receptor. Examination of GPR56 KO mice is in progress to determine if the number and/or location of GnRH cells are perturbed.

Disclosures: **F. Vilson:** None. **Y. Shan:** None. **X. Piao:** None. **S. Wray:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.16/D9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS 040449

MU Research Board

Title: Distinct roles for the adhesion molecule Contactin2 in the development and function of neural circuits in zebrafish.

Authors: *S. GURUNG, A. CHANDRASEKHAR;
Div. of Biol. Sci., Columbia, MO

Abstract: Neuronal migration and axon guidance are critical developmental processes essential for the establishment of functional neural circuits. Following migration, neurons extend axons that navigate precisely to targets to generate neural networks controlling complex motor and cognitive functions. Migrating neurons as well as navigating axons express a variety of cell surface molecules that to interact with the environment enroute to their destinations. Contactin2 (Cntn2)/Transiently-expressed Glycoprotein 1 (Tag1), a glycosylphosphatidylinositol anchored cell adhesion molecule of the immunoglobulin superfamily, is expressed in specific neuronal types during vertebrate nervous system development. Our previous studies using morpholinos suggested that *cntn2* is necessary for the migration of facial branchiomotor (FBM) neurons, a subset of branchimotor neurons that arise in rhombomere 4 of the hindbrain and migrate caudally to rhombomere 6, and that it genetically interacts with the planar cell polarity gene *vangl2* during FBM neuron migration. To validate these data, we generated loss-of-function mutations in *cntn2* using CRISPR/Cas9, and identified three alleles including two frameshift mutations generating premature stop codons (*zou20* and *zou22*) that exhibited identical phenotypes. *cntn2* expression was greatly reduced in $cntn2^{zou20}$ *and* $cntn2^{zou22}$ homozygotes, and Cntn2 protein was undetectable in both cases, suggesting these identified alleles are null. In contrast to the morphant phenotype, zygotic and maternal-zygotic (MZ) $cntn2^{-/-}$ mutants exhibited normal FBM neuron migration, suggesting either that the morphant phenotype is due to off-target effects or that there is genetic compensation from related genes in MZ mutants. Consistent with the latter, FBM neurons failed to migrate caudally in a significant fraction of $cntn2^{+/-}$; $vangl2^{+/-}$ embryos obtained from $cntn2^{-/-}$ mothers, supporting the genetic interaction observed using morpholinos. Previous studies showed that morpholino-mediated *cntn2* knockdown generated defasiculation defects in midbrain nucMLF and spinal cord Rohon-Beard (RB) axons. However, MZ $cntn2^{-/-}$ mutants exhibited no nucMLF defects. To assay for defects in the sensorimotor circuits of RB neurons in *cntn2* mutants, touch-evoked escape responses was evaluated. Wild type and mutants

larvae exhibited normal escape responses when touched on the head. In contrast, mutants were less responsive than wildtype siblings when touched in the trunk, suggestive of putative defects in RB circuits. Together, these data support distinct roles for Cntn2 in the development and function of neural circuits in zebrafish.

Disclosures: S. Gurung: None. **A. Chandrasekhar:** None.

Poster

393. Molecular Mechanisms of Neuronal and Glial Migration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 393.17/D10

Topic: A.01. Neurogenesis and Gliogenesis

Support: Israel Science Foundation (Grant 702/13 and 322/13)

Title: The spinal muscular atrophy with pontocerebellar hypoplasia gene vrk1 regulates neuronal migration through an amyloid-β precursor protein-dependent mechanism

Authors: *H. VINOGRAD-BYK[1], T. SAPIR[2], L. CANTARERO[3], P. LAZO[3], S. ZELIGSON[1], R. ORLY[2], P. RENBAUM[1], E. LEVY-LAHAD[1];
[1]Med. Genet. Inst., Shaare Zedek Med. Ctr., Jerusalem, Israel; [2]Mol. Genet., Weizmann Inst. of Sci., Rehovot, Israel; [3]Hosp. Universitario de Salamanca, Salamanca, Spain

Abstract: Spinal muscular atrophy with pontocerebellar hypoplasia (SMA-PCH) is an infantile SMA variant with additional manifestations, particularly severe microcephaly. We previously identified a nonsense mutation in Vaccinia-related kinase 1 (*VRK1*), R358X, as a cause of SMA-PCH. VRK1-R358X is a rare founder mutation in Ashkenazi Jews, and additional mutations in patients of different origins have recently been identified. VRK1 is a nuclear serine/threonine protein kinase known to play multiple roles in cellular proliferation, cell cycle regulation, and carcinogenesis. However, VRK1 was not known to have neuronal functions before its identification as a gene mutated in SMA-PCH. We show that *VRK1*-R358X homozygosity results in lack of VRK1 protein, and demonstrate a role for VRK1 in neuronal migration and neuronal stem cell proliferation. Using shRNA *in-utero* electroporation in mice, we show that Vrk1 knockdown significantly impairs cortical neuronal migration, and affects the cell cycle of neuronal progenitors. Expression of wild-type human VRK1 rescues both proliferation and migration phenotypes. However, kinase-dead human VRK1 rescues only the migration impairment, suggesting the role of VRK1 in neuronal migration is partly noncatalytic. Furthermore, we found that VRK1 deficiency in human and mouse leads to downregulation of amyloid-β precursor protein (APP), a known neuronal migration gene. APP overexpression

rescues the phenotype caused by Vrk1 knockdown, suggesting that VRK1 affects neuronal migration through an APP-dependent mechanism.

Disclosures: **H. Vinograd-Byk:** None. **T. Sapir:** None. **L. Cantarero:** None. **P. Lazo:** None. **S. Zeligson:** None. **R. Orly:** None. **P. Renbaum:** None. **E. Levy-Lahad:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.01/D11

Topic: A.09. Adolescent Development

Support: SFI/IA/1537

Title: Impact of voluntary exercise during adolescence on cognitive performance in a touchscreen operant chamber during adulthood

Authors: ***J. O'LEARY**[1], C. BROUWERS[1], N. BROSENS[1], O. F. O'LEARY[1,2], J. F. CRYAN[1,2], A. M. SULLIVAN[1], Y. M. NOLAN[1];
[1]Anat. and Neurosci., Univ. Col. Cork, Cork, Ireland; [2]Alimentary Pharmabiotic Ctr., Cork, Ireland

Abstract: Background: Adolescence is a critical period for postnatal brain maturation and thus a time for increased susceptibility to developing emotional and cognitive disorders. Exercise during adulthood has been shown to increase hippocampal neurogenesis and enhance cognition (Creer *et al*., 2010, van Praag *et al*., 2005). However, the impact of exercise during adolescence on the brain and behaviour in adulthood remains to be fully elucidated. The aim of this study was to determine the impact of voluntary exercise during adolescence on neural plasticity and performance in a hippocampal neurogenesis-dependant location discrimination task in rodents during adulthood. **Methods:** Adolescent male Sprague Dawley rats (4 weeks) were divided into a sedentary control group (n = 10) and an exercise group (n = 10). All rats were pair housed in either standard housing or with continuous access to a running wheel and were trained to use a touchscreen operant chamber. Four weeks later, rats (8 weeks) were trained to discriminate between the locations of two adjacent identical stimuli. Inter-stimulus distance was varied with a small inter-stimulus distanced probe (small separation) and large inter-stimulus distanced probe (large separation). Upon acquisition, the reward location was switched and the rats were required to complete the task. Task acquisition was assessed by trials to criteria and reversal learning was measured by the number of acquisitions/switch reacquisitions. Immunohistochemical analysis (using antibodies targeting BrdU/NeuN and PSD-95) is currently underway to determine the

impact of exercise on neural plasticity in the hippocampus and prefrontal cortex. **Results:** Rats ran an average of 1.7km per day during the adolescent period (P28-56). Voluntary exercise enhanced reversal learning in rats subjected to the location discrimination task that assessed both the small separation ($p < 0.05$) and large separation ($p < 0.05$). Exercise readily enhanced reversal learning when the task was challenging (i.e. small inter-stimulus separation) ($p < 0.05$). Interestingly, acquisition of the location discrimination task was affected by inter-stimulus distance but not exercise. **Conclusions:** Growing evidence suggests that experience during early life has a significant impact on behaviour and cognitive processes in later life. These findings suggest that exercise readily improved reversal learning, a prefrontal cortex-dependent process, particularly when the task was challenging. Investigations into the impact of exercise during early life on adult neural plasticity are ongoing.

Disclosures: **J. O'Leary:** None. **C. Brouwers:** None. **N. Brosens:** None. **O.F. O'Leary:** None. **J.F. Cryan:** None. **A.M. Sullivan:** None. **Y.M. Nolan:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.02/D12

Topic: A.09. Adolescent Development

Title: Aripiprazole sensitization: Adolescence to adulthood in the conditioned avoidance response model and pcp model.

Authors: *E. D. FREEMAN[1], J. LIN, 68526[1], C. CHOW[1], C. DAVIS[2], M. LI[2];
[1]Psychology, [2]Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: The present study investigated repeated aripiprazole (ARI) treatment and the long term consequences of ARI sensitization in adolescent male and female rats, and to determine whether ARI sensitization transfers to olanzapine (OLZ) and/or clozapine (CLZ) using two behavioral tests of antipsychotic activity. In experiment 1, male and female Sprague-Dawley rats were first trained to acquire avoidance response, and then they were tested for conditioned avoidance response (CAR) under vehicle and ARI (10 mg/kg) on P46-50. After two retraining sessions, rats were challenged with ARI (1.5 mg/kg, subcutaneously, (sc); P70), OLZ (0.5 mg/kg, sc; P73), CLZ (5 mg/kg, sc; P76) and again with ARI (1.5 mg/kg, sc; P84). On the challenge days, both male and female rats previously treated with ARI had significantly lower avoidance than the vehicle group, and females made significantly more avoidances than males in both ARI and vehicle groups. Further, on the OLZ and CLZ challenge days, prior ARI treatment seemed to increase sensitivity to OLZ exposure, however, this increase was not significant. In

study 2, we examined sex difference of ARI sensitization in the PCP-induced hyperlocomotion model. Male and female Sprague-Dawley rats (P43-47) were first treated with aripiprazole (ARI; 10 mg/kg, sc) and tested in the PCP (3.2 mg/kg, sc)-induced hyperlocomotion model for five consecutive days. Then a challenge test with ARI (3 mg/kg, sc; P76), OLZ (.5 mg/kg, sc; P80), CLZ (5 mg/kg, sc; P84) and again with ARI (3 mg/kg, sc; P84) was administered. Through the 5 drug test days, there was a sex difference response to either ARI or VEH repeated treatment during adolescence in which females demonstrated increased motor activity as compared to males overall. During the drug treatment period, repeated ARI treatment inhibited the PCP-induced hyperlocomotion, and this inhibition was progressively increased across the 5-day period in both males and females, suggesting a sensitization effect. Moreover, females demonstrated increased motor activity as compared to males. On the challenge day, rats previously treated with ARI showed a stronger inhibition of PCP-induced hyperlocomotion (i.e. sensitization) than those previously treated with vehicle. Similarly, rats also showed an ARI sensitization to OLZ or CLZ on challenge days. Collectively, results from this experiment demonstrated a sex difference in response to ARI and enhanced inhibition of PCP-induced hyperlocomotion in the animals that were pretreated with ARI as compared to controls. Specifically, it appears that ARI reduces PCP-induced increases in locomotor activity and this effect appears to be more robust in males.

Disclosures: **E.D. Freeman:** None. **J. Lin:** None. **C. Chow:** None. **C. Davis:** None. **M. Li:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.03/D13

Topic: A.09. Adolescent Development

Support: NIH AA024774

Title: Investigating the paranodal domain structure of myelinated axons in the medial prefrontal cortex of adolescent male and female rats

Authors: *E. TAVARES[1], A. SILVA-GOTAY[2], W. M. VARGAS[2], H. N. RICHARDSON[1]; [1]Psychological and Brain Sci., [2]Neurosci. and Behavior Program, Univ. of Massachusetts - Amherst, Amherst, MA

Abstract: Oligodendrocytes are glial cells that wrap segments of fatty myelin sheaths around axons in mature neurons, providing insulation and promoting faster propagation of electrical

signals along the axon. Efficient neurotransmission depends on tight organization and clustering of ion channels close to ("paranodal") and within ("nodal") the Nodes of Ranvier—the small unmyelinated sections of the axons located between segments of myelin sheaths. During adolescent development, myelin sheaths are still being formed on axons within brain regions that are involved in reward evaluation and decision-making abilities such as the prefrontal cortex. It is important to understand how myelin is formed around prefrontal axons during adolescence and whether this differs in males and females. Proliferation and cell death of oligodendrocytes is higher in females, which is thought to reflect more turnover of myelin in females. This sex difference observed in myelin turnover may affect the dynamics of axo-glial interactions that are necessary for efficient neurotransmission in myelinated axons. In the present study, we aimed to investigate whether there are sex differences in the formation of the nodes of Ranvier and surrounding structures, including the paranodal region—one of the axonal domains dependent on oligodendrocyte myelination in adolescent animals. Contactin-associated protein (caspr) is one of the cell-adhesion molecules dedicated to the formation of axo-glial contacts at the paranodal region of the nodes of Ranvier. Using immunofluorescence and confocal microscopy, we immunolabed caspr in the prefrontal cortex and forceps minor of the corpus callosum and measured nodal length (caspr gap), diameter (caspr width), and length-to-diameter ratio of mid-adolescent male and female rats. There were no sex differences in nodal length or diameter detected in any of the regions examined. However, the nodal length-to-diameter ratio in the forceps minor appears to be consistent in females (*ratio* = 1.22, *SD* = 0.01) and more variable in males (*ratio* = 1.24, *SD* = 0.14), whereas the average ratio did not differ between sexes. It is important to note that this was observed at only one time point, which did not allow us to visualize the progression of nodal and paranodal development throughout adolescence. Future studies with more developmental time points might help uncover the dynamics of nodal and paranodal development in the adolescent prefrontal cortex.

Disclosures: E. Tavares: None. **A. Silva-Gotay:** None. **W.M. Vargas:** None. **H.N. Richardson:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.04/D14

Topic: A.09. Adolescent Development

Support: nn

Title: chronic adolescent stress impairs spatial working memory in adult female rats

Authors: *R. MORANO;
Psychiatry, Univ. of Cincinnati, Cincinnati, OH

Abstract: During adolescence in rodents, the central nuclei that regulate the hypothalamic-pituitary-adrenocortical (HPA) axis, including the amygdala, hippocampus and prefrontal cortex are continuing to develop. These brain regions are densely populated with glucocorticoid receptors making them susceptible to plastic changes due to chronic glucocorticoid exposure. Our lab has previously shown that late adolescence is a period of increased vulnerability to the effects of chronic stress (PND 50 at initiation of stress) as indicated by somatic indices and increased basal corticosterone in male rats. Our lab has also shown that adult female rats (PND 101) exposed to chronic stress during the late adolescent period (PND 45-58) have a blunted HPA axis response to a novel stressor and increased immobility in the forced swim test. In the current study, male and female Sprague-Dawley rats were exposed to three weeks of chronic variable stress during adolescence with a focus on late adolescence. Our hypothesis for this experiment was that adolescent chronic stress would lead to enduring deficits in cognition and executive behavior in adult males and females. Separate cohorts of these animals were tested in adulthood for fear conditioning and delayed spatial win-shift (DSWS) to assess deficits in behavioral flexibility caused by adolescent chronic stress. Neither males nor females stressed in adolescence showed differences in the conditioning or extinction of the conditioned stimulus when compared to previously unstressed controls. However, the females exposed to adolescent stress exhibited a decrease in freezing to the conditioned stimulus when the recall of extinction was tested. Adolescently stressed females made more across phase and within phase errors on day 1 of testing and more within phase errors on day 3 of testing in the DSWS when compared to no stress controls. In contrast, there was a main effect of chronic stress during adolescence to reduce the overall errors in the DSWS in the adult males. These results suggest that the long-term effects of chronic adolescent stress are sex-specific, whereby females are more susceptible to altered stress responsiveness and impaired behavioral flexibility.

Disclosures: R. Morano: None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

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OD P51OD011132

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MH096773

K99/R00 MH091238

Title: Effect of pubertal delay and social stress on hippocampal connectivity in adolescent female rhesus macaques.

Authors: *M. PINCUS[1], J. GODFREY[1], E. FECZKO[2], E. EARL[2], C. KELLY[3], M. WILSON[1], D. FAIR[2], M. SANCHEZ[1];
[1]Emory Univ., Atlanta, GA; [2]Oregon Hlth. & Sci. Univ., Portland, OR; [3]Trinity Col., Dublin, Ireland

Abstract: The hippocampus plays a pivotal role in fear learning and regulation by contextually gating fear responses through its connections to the amygdala and medial prefrontal cortex (mPFC). Chronic stress impairs extinction-related mPFC-hippocampus long-term potentiation in animal models, and exposure to stressors during military service has been found to be associated with enhanced functional coupling (FC) in this circuitry. Adolescence is marked by enhanced fear learning, suggesting that fear learning circuitry may be affected by pubertal development. In support of this view, the hippocampus has been shown to undergo structural and functional changes during puberty. Given that stress and puberty may both modulate fear learning circuitry, we examined whether the development of hippocampal circuits are affected by chronic psychosocial stress and puberty-induced elevations of estradiol (E2) in adolescent female macaques. As part of a longitudinal study using social subordination as a model of psychosocial stress, female macaques were randomly assigned to either experience puberty spontaneously (n=34), or receive monthly injections of the GnRH agonist Lupron from 14-36 months of age to delay puberty (n=36). Resting state fMRI scans were acquired post-pubertally (43-46 months) and in a preliminary analysis we tested for effects of rank, Lupron treatment, and pubertal timing on hippocampal functional connectivity (FC) with mPFC and amygdala using linear regression. Because stronger mPFC-hippocampal FC was associated with delayed pubertal onset (BA32: β = 0.046, 95% CI [0.003, 0.088]; BA25: β = 0.077, 95% CI [0.025, 0.127]), we ran the statistical models controlling for pubertal onset, and found that Lupron-treated females had weaker mPFC-hippocampal FC than untreated controls (BA32: β = -0.424, 95% CI [-0.768, -0.004], BA25: β = -0.690, 95% CI [-1.289, -0.228]), suggesting that Lupron effects may be due to lower E2 levels even during the pre-pubertal period. Lower social rank was associated with higher mPFC-hippocampal FC (BA32: β = 2.664, 95% CI [0.472, 3.958], BA25: β = 3.200, 95% CI [0.455, 4.197]), which may reflect a greater stress load or an adaptation for improved contextual fear responses in an unpredictable social environment. Taken together, these data suggest that E2 levels, pubertal timing and chronic stress impact the maturation of mPFC-hippocampal circuitry during adolescence, which may have implications for contextual fear learning and regulation. Further analysis will assess whether the observed mPFC-hippocampal FC differences are

associated with variability in emotion regulation behaviors and stress physiology in adolescent females.

Disclosures: **M. Pincus:** None. **J. Godfrey:** None. **E. Feczko:** None. **E. Earl:** None. **C. Kelly:** None. **M. Wilson:** None. **D. Fair:** None. **M. Sanchez:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.06/D16

Topic: A.09. Adolescent Development

Title: Role of CB1 receptor signaling in the regulation of afferent-evoked plasticity in the prefrontal cortex *In vivo*

Authors: *H. M. MOLLA, D. R. THOMASES, K. Y. TSENG;
Dept. of Cell. and Mol. Pharmacol., The Chicago Med. Sch. At RFUMS, North Chicago, IL

Abstract: The adolescent maturation of the prefrontal cortex (PFC) is characterized by a number of structural and functional changes. Developmental augmentations of both GABAergic and glutamatergic transmission contribute to the maturation of cognitive function and the normative integration of afferent inputs. Basolateral amygdala (BLA) afferents evoke a non-developmentally regulated prefrontal LTP, while ventral hippocampus (vHipp) afferents elicit an age-dependent prefrontal LTD. Coinciding with these changes is a developmental downregulation in the expression of cannabinoid receptor-1 (CB1R) mRNA in the medial PFC. However, the functional significance of this downregulation of CB1Rs and its role in prefrontal afferent processing is not fully understood. Here we assessed the role of CB1R mediated transmission in the regulation of prefrontal long-term plasticity evoked by the vHipp and the BLA in both adolescent (P30-40) and adult (P>60) rats. We found that local prefrontal application of the CB1R inverse agonist AM251 (4 μM) does not affect the normal expression of vHipp-evoked prefrontal LTD. In contrast, a transient facilitation of BLA-evoked LTP is observed in adult rats. This input-specific sensitivity indicates CB1R tone regulates the PFC response to BLA inputs. Moreover, the facilitation of BLA-evoked prefrontal LTP by AM251 was found to be age-dependent, with no observable effects in the adolescent PFC, indicating a change of CB1R transmission during adolescence. Interestingly, local infusion of the CB1R agonist Win 55,212-2 (3 μM) both attenuates the expression of BLA-evoked prefrontal LTP and transiently enhances the expression of vHipp-evoked prefrontal LTD. Taken together these data indicate CB1 transmission developmentally regulates prefrontal long-term plasticity in an input-specific manner.

Disclosures: **H.M. Molla:** None. **D.R. Thomases:** None. **K.Y. Tseng:** None.

Poster

394. Adolescent Development: Animal Models II

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Program#/Poster#: 394.07/D17

Topic: A.09. Adolescent Development

Support: NIH Grant DA034185

Title: Neuroimmune signaling in the nucleus accumbens underlying the adolescent critical period for drugs of abuse

Authors: ***A. M. KOPEC**, S. C. SWEAT, N. R. AYRE, S. D. BILBO;
Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: During adolescence, the reward circuitry in the brain is undergoing rapid development, rendering it particularly vulnerable to external influences during this time, including drugs of abuse. Adolescent drug users have poor adult outcomes, including addiction-related behaviors and other mental health disorders. Microglia, the resident immune cells of the brain, can respond directly to opioid drugs (Hutchinson et al. 2010, Schwarz et al. 2013), and increasing evidence points to a critical role for these cells in addiction. Interestingly, male rats that receive morphine during a short period in adolescence (postnatal days 37-54; P37-54) have increased rates of reinstatement (relapse) later in life, and this effect is contingent on neuroimmune signaling in the nucleus accumbens (NAc) during adolescence (Schwarz and Bilbo 2013). However, the mechanisms by which neuroimmune signaling persistently alters the neural circuitry of the NAc and thus lastingly alters behavior, remain unknown. To begin to understand how neuroimmune signaling may be dysregulated by adolescent drug use, we first sought to characterize the role of neuroimmune signaling throughout the natural development of the NAc. Tissue was collected from male and female rats at P20, P30, P38, and P54 for immunohistochemical, gene expression, and protein analyses. The data indicate that neuron-microglia communication brokers, including fractalkine and CD200 signaling pathways, may be regulated in a sex-specific manner during NAc development. Interestingly, there may also be sex-specific morphological regulation of microglia during development. Collectively, these data suggest that the development and/or regulation of neuroimmune signaling in the NAc may be sexually dimorphic during the adolescent period. These results will be of considerable interest to investigate further, as they may suggest the need for differential treatment of males and females suffering from addiction.

Disclosures: **A.M. Kopec:** None. **S.C. Sweat:** None. **N.R. Ayre:** None. **S.D. Bilbo:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.08/D18

Topic: A.09. Adolescent Development

Title: Differential effects of paradoxical sleep deprivation on adolescent and adult mice

Authors: ***L.-H. TUAN**[1], L.-J. LEE[1,2,3];
[1]Grad. Inst. of Anat. and Cell Biol., [2]Grad. Inst. of Brain and Mind Sci., [3]Ctr. for Neurobio. and Cognitive Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Sleep insufficiency has become a serious health issue. In the modern society, most of the people, including the adolescence, do not obtain enough sleep.Since adolescence is a critical period for brain development, the consequences of insufficient sleep during adolescence should be concerned. In this study, we examined the acute effects of 72-hour paradoxical sleep deprivation (SD). Five weeks old and 10-12 weeks old male C57/BL6 micewere used. The two time points were chosen to represent the periods of adolescence and adulthood, respectively.SD for 72 hours were conducted using modified multiple platform method, in which mice were put in on small platforms(3 cm in diameter) surrounded by water and allowed to move freely from one platform to another. For mice kept in the home cage and on big platforms, sleep time was not limited and used as controls. All three groups of mice were kept under 12 h/12h light/dark cycle.Mice of SD and control groups were examined in behavioral, neurochemical and histological aspects. Our results showed that the short-term spatial memory, examined by Y-maze spontaneous alternation test,was affected by 72-hour paradoxical SD in adolescent mice but not in adult mice. The complexity of granule cells in dentate gyrus (DG)was reduced after SD in adolescent but minimal changes were observed in adult animals.There was an increase of spine density in DG granule cell after 72-hour paradoxical SD in adolescent and notin adult SD animals. Our results indicated the adolescent mice are relatively more sensitive to SD than the adult mice. Sufficient sleep during adolescent period is important for cognition and the brain development.

Disclosures: **L. Tuan:** None. **L. Lee:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.09/D19

Topic: A.09. Adolescent Development

Title: Arginine vasopressin expression mediates paternal influence on female offspring aggression in Peromyscus californicus

Authors: *C. YOHN, A. LEITHEAD, E. A. BECKER;
Psychology, St. Joseph's Univ., Philadelphia, PA

Abstract: Paternal investment in the biparental and territorial California mouse (*Peromyscus californicus)* boosts pup survival and shapes adult offspring social behavior. Male offspring that experience high levels of paternal retrievals during postnatal development exhibit increased aggressive behavior and arginine vasopressin immunoreactivity (AVP-ir) in the bed nucleus of the stria terminals (BNST) in adulthood. Since offspring experience a transient surge in Testosterone (T) in response to paternal retrievals and AVP is androgen dependent during development, we posit these long-term changes in brain and behavior are likely a consequence of the elevated T levels. While this powerful relationship between paternal care and offspring development has been demonstrated in males, the father-daughter relationship has only recently been examined. Similar to male offspring, paternal retrievals induce both transient increases in T during early development and aggressive behavior in adult female *Peromyscus californicus* offspring. In the present study, we investigated whether similar neural mechanisms underlie the behavioral transmission between fathers and daughters. Since females experience an increase in T similar in magnitude to male offspring, and because AVP is associated with adult female aggression, we hypothesized that AVP expression in the female brain will change in response to paternal high levels of paternal care. To explore this aim, female *Peromyscus californicus* offspring were randomly assigned to either a high or low care condition during postnatal days 15-21 when paternal retrieval behavior is the highest. At adulthood, focal animals were randomly assigned to either one resident-intruder test or to immunohistochemical analysis of AVP within the social regions of the brain. Our results support previous studies showing that high paternal care females have shorter attack latency’s than females from the low paternal care offspring. Additionally preliminary data suggests that females from the high care group have higher AVP-ir expression in the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus (PVN) than offspring from the low care group with a positive relationship emerging between BNST and PVN. Thus, AVP is a likely mechanism underlying the transmission of aggressive behavior between father and daughter. Additionally, given the role of the PVN in the HPA response, our data illustrates a paternally induced sensitivity to stressors in female offspring.

Disclosures: **C. Yohn:** None. **A. Leithead:** None. **E.A. Becker:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.10/D20

Topic: A.09. Adolescent Development

Support: F32MH110107

NIMH R01MH087542

UC Berkeley Center on the Developing Adolescent

Title: Gonadal steroids at puberty drive organizational effects on inhibitory neurotransmission in the mouse frontal cortex.

Authors: *D. PIEKARSKI[1], J. R. BOIVIN[2], A. W. THOMAS[1], L. WILBRECHT[1]; [1]Univ. of California, Berkeley, Berkeley, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: The age at puberty onset is advancing in the developed world (Aksglaede et al., 2009; Euling et al., 2008) and early puberty is associated with heightened risk for development of neuropsychiatric illness (Deardorff et al., 2007; Whittle et al., 2012; Deardorff et al. 2013; Graber, 2013). Pubertal onset also potentially plays a causal role in the closure of juvenile forms of plasticity that are permissive for dramatic reorganization of cortical connectivity and function (Lenneberg, 1967; Doupe and Kuhl, 1999). Inhibitory neurotransmission is thought to play a major role in regulating cortical sensitive periods in primary sensory cortex (Werker and Hensch, 2015) and may play a similar role in association cortex (Piekarski et al., in review). We have found that inhibitory neurotransmission onto pyramidal neurons matures in the mouse frontal cortex through the adolescent period (Vandenberg et al., 2015). To investigate the effects of pubertal steroids on the maturation of the frontal cortex, we developed a mouse model of earlier puberty in female mice using estradiol and progesterone injections. We also delayed pubertal onset using ovariectomy. Using slice electrophysiology to probe the maturation of neurotransmission in the frontal cortex layer 2/3 pyramidal neurons, we found miniature inhibitory post synaptic currents (mIPSCs), a measure of synaptic inhibition, increase in frequency and amplitude across adolescent development. The rise in mIPSC frequency was shifted to an earlier timepoint after gonadal steroid injection and prepubertal ovariectomy prevented this increase. Hormone treatment after ovariectomy was sufficient to rescue the

mIPSC frequency increase. Postpubertal ovariectomy, however, had no effect on mIPSC frequency. In control experiments in primary somatosensory cortex, gonadal steroid related changes in mIPSCs were not observed. These data suggest that gonadal steroids have an organizational effect on the maturation of inhibitory neurotransmission in the frontal cortex in the female brain. These data provide a potential mechanism by which puberty onset may affect the increased vulnerability for depression and anxiety observed after puberty in girls and alter the capacity for plasticity in association cortex.

Disclosures: D. Piekarski: None. **J.R. Boivin:** None. **A.W. Thomas:** None. **L. Wilbrecht:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.11/D21

Topic: A.09. Adolescent Development

Support: DPU-RFUMS Pilot Grant

Title: Social defeat stress during adolescence impairs the maturation of GABAergic function in the adult prefrontal cortex

Authors: *E. FLORES-BARRERA[1], D. R. THOMASES[1], A. CABALLERO[1], J. S. CARTER[2], K. E. GRANT[2], J. A. ROSENKRANZ[1], K. Y. TSENG[1];
[1]Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]Dept. of Psychology, DePaul Univ., Chicago, IL

Abstract: Early life exposure to emotionally salient stressors is thought to contribute to the development of maladaptive behavioral patterns later in life, many of which are associated with aberrant functioning of prefrontal-mediated cognitive processing. However, the mechanisms underlying such developmental disruption remain unclear. Here we asked whether a disruption of prefrontal maturation could underlie the enduring cognitive impairments resulting from repeated social stress during adolescence (from postnatal day -P- 32 to P38). Using electrophysiological measures, we found that the normal inhibitory control of afferent drive is lacking in the adult prefrontal cortex (PFC) of rats that underwent adolescent social defeat. Results from local field potential recordings and ventral hippocampal stimulation revealed that the pattern of prefrontal response observed in the social defeat stress group resembles that seen in the immature PFC of naïve juvenile rats. At the cellular level, such inhibitory disruption in the PFC was associated with a state of increased AMPA/GABA ratio in layer V pyramidal neurons,

mainly due to a selective downregulation of local GABAergic transmission. A similar dysregulation of prefrontal GABAergic function was observed in rats that underwent social defeat stress during the P42-48 adolescent period. However, no deficits in PFC inhibition were observed when the repeated social defeat stress was presented during late adolescence (i.e., P50-55) or adulthood (P75-80). Collectively, these results indicate that social defeat stress during early and mid-adolescence had the most potent and enduring impact on PFC function and maturation, in particular at the level of local GABAergic function. As fine-tuning of PFC output and behavior are highly dependent on the activity of local inhibitory interneurons, impaired GABAergic maturation resulting from social defeat stress during adolescence is expected to elicit enduring deficits in prefrontal-dependent cognitive functions.
Supported by DPU-RFUMS Pilot Grant

Disclosures: **E. Flores-Barrera:** None. **D.R. Thomases:** None. **A. Caballero:** None. **J.S. Carter:** None. **K.E. Grant:** None. **J.A. Rosenkranz:** None. **K.Y. Tseng:** None.

Poster

394. Adolescent Development: Animal Models II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 394.12/D22

Topic: A.09. Adolescent Development

Support: R01AG045380

Title: Hedonic reward consumption is elevated in male, but not female, adolescent rats

Authors: ***A. T. LIU**[1], Y. CUI[1], N. P. MURPHY[2], N. T. MAIDMENT[2], S. B. OSTLUND[1]; [1]Dept. of Anesthesiol. and Perioperative Care, UC Irvine, Irvine, CA; [2]Hatos Center, Dept. of Psychiatry and Biobehavioral Sciences, Semel Inst. for Neurosci., UCLA, Los Angeles, CA

Abstract: There is growing evidence that decision-making is profoundly altered during adolescence, resulting in excessive risk-taking behavior and heightened vulnerability to drug addiction. Determining how adolescent neural development impacts more fundamental aspects of reward processing will be key to improving our understanding of risky adolescent behavior. The current study investigated this issue by characterizing microstructural patterns of voluntary reward consumption in peri-adolescent and adult rats. We first conducted a quasi-longitudinal experiment tracking consumption of a highly palatable sweetened condensed milk solution (SMC, 10%) over several weeks in non-deprived male and female rats, beginning at PD 30 (covering adolescence) or PD 60 (covering early adulthood). We found that male, but not female, adolescent rats showed a pronounced increase in SCM intake (normalized for bodyweight)

around PD 50 (roughly corresponding to male puberty). This effect was associated with various alterations in the microstructure of licking behavior, with males showing larger mean lick volumes, as well as longer and more frequent bouts of licking early in the session, prior to the induction of satiety. To more precisely target hedonic aspects of feeding, we conducted a second experiment examining saccharin consumption in male adolescent and adult rats. We found that, here too, male adolescent rats showed elevated reward consumption, particularly early in test sessions, prior to satiety, an effect that appeared to be driven by longer bouts of active licking behavior. Ongoing experiments are examining whether this heighted reward processing during male adolescence is associated with alterations in reward-related neural activity.

Disclosures: A.T. Liu: None. **Y. Cui:** None. **N.P. Murphy:** None. **N.T. Maidment:** None. **S.B. Ostlund:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.01/D23

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: OTKA grant, No. 111990

RFBR grant, No. 12-04-01510.

Title: Neuronal signal molecules in developing and adult Dreissena, an environmental biofouling mollusk

Authors: *I. BATTONYAI[1], E. E. VORONEZSHKAYA[2], A. OBUKHOVA[2], L. P. NEZLIN[2], K. ELEKES[1];
[1]MTA Ctr. For Ecolog. Res., Balaton Limnol. Inst., Tihany, Hungary; [2]Inst. of Developmental Biology, Russian Acad. of Sci., Moscow, Russian Federation

Abstract: Zebra mussel (*Dreissena polymorpha*) is an invasive molluscan species, appearing in Lake Balaton (Hungary) by 1930 and becoming soon thereafter its ruling bivalve. The question arises what might be the basis of this successful invasive behavior. Apart from pure ecological considerations, one, maybe a less considered possibility is the role of early sensory elements capturing chemical signals, followed by proper adaptive responses. Therefore, as a first attempt, we have investigated the development of the nervous system of trochophore and veliger *Dreissena* larvae, with special attention to their signal molecule (serotonin [5-HT] and FMRFamide [Fa]) content. On the other hand, we have also investigated the innervation of the

byssus retractor muscle (BRM), which is responsible for anchoring the animals to different surfaces, with special emphasis to 5-HT, Fa and choline acetyltransferase (ChAT) containing elements, respectively. Immunohistochemical techniques combined with confocal laser microscopy were used for visualization. The first 5-HT containing cells were found in sensory cells of the apical organ (AO) as early as in 16-18 hours post-fertilization free living trochophores. It was followed by the appearance of a caudal and a stomach sensory cell at 48-60 hours veliger stage. A similar developmental scenario was characteristic for the Fa-immunreactive (IR) system, starting however later by 32 hours. Pharmacological experiments proved the role of 5-HT in accelerating larval swimming activity, paralleled with enhanced relative immunofluorescence intensity. Increased salinity evoked similar changes in the 5-HT and Fa immunoreactivities. Our findings suggest the suitability of the larval nervous system and behavior of *Dreissena* to test the effect of different environmental cues to better understand the neuroethological background of the invasive behavior. The innervation pattern of the BRM was characterized by varicose networks formed by both 5-HT- and Fa-IR fibers with branching into thin fibers supplying the finger-like extrusions of the BRM. ChAT immunoreactivite fine processes was also present along the finger-like extrusions, referring to additional cholinergic regulation of muscle function. It is assumed that the BRM stands under aminergic and peptidergic influence, modulating cholinergic transmission. Our results provide a good basis for further physiological and pharmacological characterization of the BRM as a possible target of different environmental cues.

Disclosures: I. Battonyai: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); OTKA GRANT No. 111990; RFBR GRANT No. 12-04-01510. **E.E. Voronezshkaya:** None. **A. Obukhova:** None. **L.P. Nezlin:** None. **K. Elekes:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.02/D24

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Beta Beta Beta Research Grant

Title: Behavioral pharmacology of planaria (Giardia) as a model for glutamate excitotoxicity.

Authors: M. SCRIBNER, *B. R. MILLER;
Texas Wesleyan Univ., Fort Worth, TX

Abstract: Planaria (*Girardia*) are a useful animal model in neuropharmacology as their neurological features are strikingly similar to human and other vertebrate neuronal physiology. One example is that glutamate serves as the primary excitatory neurotransmitter in humans and planaria. Importantly, in humans, neuronal over excitation with glutamate results in neurodegeneration; a key feature in a number of neurological diseases. To date, the effects of glutamate have yet to be extensively studied in planaria, which can serve as a model organism for drug discovery. The purpose of this study is to establish a glutamate concentration-response curve for planarian behaviors. This endeavor was accomplished by observing planaria in increasing glutamate concentrations and identifying four behaviors. Here, single planarians were placed into petri dishes with a marked 0.5 x 0.5 cm grid and video-recorded for 5 minute intervals. There were 11 tested glutamate concentrations ranging from 0.001 mM to 250 mM. Four behaviors were analyzed; two standard planarian behavioral metrics, line crosses and C/S-shaped convulsions, as well as, two newer metrics, crenellations and corkscrews. The most sensitive metrics were C/S-shaped convulsions (at 3.0 mM and greater) and corkscrew (at 1.0 mM and greater). Therefore, we suggest that planaria are a useful model organism for evaluating glutamate excitotoxicity.

Disclosures: M. Scribner: None. **B.R. Miller:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.03/D25

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Requirement of IP_3R function and SOCE in dopaminergic interneurons for *Drosophila* flight

Authors: *S. SADAF[1], S. P. SANE[2], G. HASAN[3];
[1]Natl. Ctr. For Biol. Sci., Bangalore, India; [2]Natl. Ctr. for Biol. Sci., Bangalore, India; [3]Natl. Ctr. for Biol. Scirnces, Bangalore, India

Abstract: Neural circuits respond to various stimuli, integrate the signals and give rise to accurately tuned animal behavior. The environmental cues are detected by cells and are further relayed down through specific sets of participating cells in the form of biochemical signals. One of the robust signaling molecules is calcium (Ca^{2+}), which acts as a second messenger. The neural circuits are shaped and modulated during different developmental stages and Ca^{2+} signals play an important role in their development, maintenance and modulation.
In bilaterally symmetric animals, behaviors such as movement of limbs in mammals or

movement of wings in fruit flies (*Drosophila melanogaster*) are dictated by specific sets of symmetric as well as asymmetric neural circuits (1). In *Drosophila*, for example, the left and the right wings have their own sets of motor machinery including motor neurons and their target muscles. Neurotransmitters and neuromodulators form the functional connectivity between the various components of the neural circuit.
In our previous study, we had investigated the functional connectivity between dopaminergic neurons in the second thoracic segment. The a-a' dopaminergic connect to the ventral unpaired medial (VUM) neuron. The VUM sends axonal projections to the b1Mns and together this circuit mediates coordinated bilateral wing movements (2).
IP_3R, dSTIM and Orai, are the three major components of the GPCR activated IP_3 signaling pathway and contribute to intracellular Ca^{2+} signals. The role of the IP_3 signaling components in dopaminergic neurons was shown in our recent paper (3).
This study focuses on the requirement of IP_3 signaling and store operated calcium entry in the T2 dopaminergic neurons for flight and bilateral wing movements.
We find that the IP_3R, dSTIM and Orai function is required in dopaminergic neurons for free flight in the cylinder drop test. IP_3R function and store operated Ca^{2+} entry (SOCE) is required in dopaminergic neurons to mediate bilateral coordination of wing movements during flight initiation and cessation. Furthermore, this required can be narrowed down to a paired set of dopaminergic neurons in the adult thoracic ganglion. The IP_3 signaling is mediated through the GPCR, mAcR, for flight and wing movement coordination
1) Concha M.L. et al., Nat Rev Neurosci, 13(12):832-43, 2012.
2) Sadaf S. et al., Curr Biol, 25(1):80-6, 2015.
3) Pathak T. et al., J Neurosci, 35(40):13784-99, 2015.

Disclosures: **S. Sadaf:** None. **S.P. Sane:** None. **G. Hasan:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.04/D26

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSERC grant DIS-0000065 (to I.A.M.)

NSERC grant RGPAS429437 (to I.A.M.)

Title: A diurnal rhythm in head histamine in wild-type and white *Drosophila*

Authors: *J. BORYCZ[1], J. A. BORYCZ[1], I. A. MEINERTZHAGEN[1,2];
[1]Dept. Psychology & Neurosci., [2]Dept. Biol., Dalhousie Univ., Halifax, NS, Canada

Abstract: Histamine (HA) is a photoreceptor neurotransmitter in arthropods. Most released HA is recycled via conjugation with β-alanine to form β-alanylhistamine (carcinine), a process mediated by Ebony in the epithelial glia that surround photoreceptor terminals in the first optic neuropil, and the carcinine is then transported back to photoreceptors where Tan mediates its hydrolysis to liberate HA and β-alanine. We have previously reported that light-induced HA-release is potentiated in the mutant chalky of *Calliphora erythrocephala,* most probably because in the absence of screening pigments more photoreceptors are overstimulated by scattered light than in wild-type. We have also shown that head histamine content is reduced roughly by half in the white-eyed mutants of different species e.g. *Drosophila*, *Musca*, *Calliphora* and *Sarcophaga.* In our observations the *Drosophila* mutant *white*^{1118} (w^{1118}) kept for 3 days in constant light had its head histamine decreased by almost 40% whereas the same treatment did not alter head HA in the wild-type (Borycz et al. 2008). Here, we wanted to analyse whether there is a diurnal rhythm in head histamine in wild-type *Drosophila* and if this is affected in the mutant *white*. Animals were kept at 24ºC at 12/12 h light-dark cycle. They were collected in 6-hour intervals, starting from the beginning of the light phase. During the light phase head HA increases slowly in the wild-type reaching a maximum at 12 hours of light, an increase by 18% over the lowest HA content observed at the beginning of the light phase. In contrast in the head of the w^{1118} HA slightly decreases by 5% at midday and by 12% at the end of the light phase. In the dark phase HA in the head of wild-type flies decreases slowly, losing the 18% which was gained during the light phase. In contrast HA increases during the dark phase in the head of w^{1118} mutant flies, restoring the 12% lost during the light phase. Our results demonstrate that HA content oscillates daily in the fly's brain. Lack of the White transporter in w^{1118} reverses this rhythm and diminishes the fly's ability to efficiently recycle and/or synthesize HA during the light phase, although the loss can be recovered in darkness by the w^{1118} mutant.

Disclosures: J. Borycz: None. **J.A. Borycz:** None. **I.A. Meinertzhagen:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.05/D27

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Daphne Jackson Fellowship

Title: Temporal profiling of the phosphorylation of CaMKII at Threonine 286 & Threonine 305 following classical conditioning

Authors: *H. WAN[1], G. KEMENES[2];
[2]Sussex Neurosci., [1]Univ. of Sussex, Brighton, United Kingdom

Abstract: CaMKII is considered a 'memory' molecule and has two important phosphorylation sites, threonine 286 (T286) and threonine 305 (T305). The activation of CaMKII, due to phosphorylation at T286 (pT286), is known to be pivotal for both memory acquisition and initiation of synaptic plasticity, the substrate for learning as well as late memory consolidation. Following the initial phosphorylation at T286, there is a second wave of CaMKII phosphorylation at T305 (pT305). CaMKII inhibitory phosphorylation at T305 functions to inactivate CaMKII and is involved in synaptic metaplasticity and refining of memory and has recently been reported to be necessary for on-going memory consolidation. Despite a plethora of studies on CaMKII over the last 3 decades, it is still unclear when CaMKII becomes inactivated following activation or if the activation-inactivation process is repeated several times during memory consolidation. Here we present a temporal profile of the phosphorylation of CaMKII at T286 and T305 following classical conditioning in *Lymnaea*, an established invertebrate model. Following single-trial classical conditioning with amyl acetate as the conditioned stimulus and sucrose as the unconditioned stimulus, the buccal and cerebral ganglia that are important for learning and memory were collected at hourly intervals after training. The samples were subjected to western blotting, with specific antibodies for CaMKII, pT286-CaMKII and pT305-CaMKII. We found that the changes in the levels of pT286-CaMKII and pT305-CaMKII show an opposing trend: pT286-CaMKII is swiftly increased, at 2 min post conditioning, and gradually decreases thereafter, returning to the baseline level at 6h post conditioning. pT305-CaMKII, however, displays changes at a much slower pace by gradually increasing from 1h onwards. The enhanced pT305, however, does not return to the baseline level, rather, it is still maintained at 6h post conditioning. Temporal profiling of pT286-CaMKII and pT305-CaMKII for the time points after 6 h is ongoing. The completed study will, for the first time, reveal the detailed temporal characteristics of CaMKII phosphorylation during memory consolidation, and open new avenues for research on later memory processes, i.e. memory reconsolidation and storage. These results will also have wider implications for studies of other serine/threonine kinases.

Disclosures: H. Wan: None. **G. Kemenes:** None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.06/D28

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: HHMI

Point Loma Nazarene University

PLNU Research Associates

Title: Dynamics of change of pattern of serotonin-containing neurons in the terminal abdominal ganglion of a tenebrionid beetle during metamorphic development.

Authors: *R. C. ELSON;
Point Loma Nazarene Univ., San Diego, CA

Abstract: During metamorphic development of the tenebrionid beetle, *Zophobas morio*, the number and type of neurons in the terminal abdominal ganglion that show serotonin-like immunoreactivity (SLI) changes in a complex and dynamic fashion. In the larval stage, the hindgut is innervated by two pairs of serotonin-containing efferent neurons; the segmental system of serotonin interneurons is present in incomplete form; and three further pairs of serotonin interneurons are present. As metamorphic development proceeds, one pair of efferent neurons and two of the extra pairs of interneurons lose SLI. The segmental system of serotonin interneurons develops and reaches completion as a pattern of 1-3 pairs of neurons per neuromere depending on segment identity. The temporal changes in the number and type of neurons involve gain, loss, or persistence of SLI. In most instances, these changes seem to arise from up- or down-regulation of serotonin content in existing neurons. These dynamics correlate with the reported temporal variation of hemolymph titer of the molting hormone, 20-hydroxy ecdysteroid.

Disclosures: R.C. Elson: None.

Poster

395. Invertebrate Neurotransmitters

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 395.07/D29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Sam Taylor Fellowship

Title: Automated behavioral pharmacology assay using planaria (Girardia) in a 3-D printed apparatus.

Authors: *D. POE[1], A. CORNWALL[2], M. TAYLOR[2], B. MILLER[2];
[1]UNT Syst. Col. of Pharm., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [2]Texas Wesleyan Univ., Fort Worth, TX

Abstract: Several key features make planaria an effective biological model for neuroscience studies. Namely, planaria exhibit cephalization and a nervous system that has remarkable similarities to mammalian species. Planarian membranes are highly permeable to pharmacological agents which allows for tight dosing and concentration controls. Additionally, planaria are inexpensive making them suitable for use in a wide range of settings including undergraduate studies. One challenge of using planaria as behavioral models is that often the data are hand-coded making the task labor-intensive and heavily dependent on inter-coder reliability. To address these challenges, we constructed an apparatus which incorporates several off-the-shelf components and novel 3-D printed components into an automated system. The system consists of five lanes which may be simultaneously managed by a single operator. Each lane is a modular, self-contained testing environment which can be filled with pharmacologically neutral or active media depending on the testing protocol. A single planarian is placed at the top of a lane and exposed to an ultraviolet-light stimulus. The planarian's photophobic taxis away from that stimulus is monitored by two infrared beam-break sensors and these data are relayed by an Arduino microcontroller to either the operator's computer or to a secure digital (SD) non-volatile memory card. Using this apparatus, we have found that completing a protocol where n = 30 can be achieved in less than two hours and that we are able to plot a glutamate dose-response in concentrations as low as 0.01 μM. Indeed, this system allows for high-throughput, high-accuracy testing using the planaria model in addition to being readily scalable, easy-to-use and cost-efficient. The system is ripe for implementation in any laboratory doing behavioral neuropharmacology with planaria as well as in undergraduate learning laboratories.

Disclosures: D. Poe: None. **A. Cornwall:** None. **M. Taylor:** None. **B. Miller:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.01/D30

Topic: B.02. Ligand-Gated Ion Channels

Support: MRC

Title: Dysfunctional NMDA receptors affect excitatory transmission causing neurological disease

Authors: *L. FEDELE[1], R. J. HARVEY[2], T. G. SMART[1];
[1]Neuroscience, Physiol. and Pharmacol., Univ. Col. of London, London, United Kingdom; [2]UCL Sch. of Pharm., London, United Kingdom

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that together with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate (KA) receptors mediate the vast majority of fast excitatory neurotransmission in the central nervous system. Given this role, any dysfunction to excitatory neurotransmission is likely to have a severe impact on brain physiology and subsequent behaviour. Recently, *de novo* mutations have been reported in several subunits (GluN2) of the NMDAR for patients exhibiting a number of neurological disorders such as: autism spectrum disorders, epilepsies, and intellectual disabilities characterised by marked cognitive impairments[1]. Here, we have used a combination of techniques based on structural modelling, site-directed mutagenesis and electrophysiology to deduce how selected mutations located to the NMDAR ion channel and to the GluN2 subunit N-terminal domain are affecting NMDAR function. We hypothesized that these missense mutations could be correlated with the phenotypes of the patients and we therefore sought to understand the molecular mechanisms that underlie the clinical disease profiles.

We developed a complete tetrameric structural model of the NMDAR (GluN1-GluN2A,B) based upon the recently reported crystal structures of the NMDARs[2,3]. By using more than one such template we were able to include several loop regions of the NMDAR in one structure, some of which were deleted in the crystallography studies. This facilitated the identification of the locations for mutated (pathogenic) residues and helped us deduce their potential impact on neighbouring amino acids and receptor structure. The functional effects of several mutants of the GluN2 subunit resulted in both loss-of-function and gain-of-function phenotypes. The most profound phenotypes were used for expression studies in cultured neurons revealing how these mutations can compromise excitatory synaptic transmission in patients.

Overall, our results suggest that some of the NMDAR mutations can be correlated with the phenotypes of the patients. In addition, our analyses will be beneficial in indicating what pharmacotherapeutic interventions are most likely to be successful as potential treatments.

References

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Disclosures: L. Fedele: None. **R.J. Harvey:** None. **T.G. Smart:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.02/D31

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 1R15AG045820-01A1

George Mason University Summer Research Award

Title: Neuromorphological characterization of CA1 pyramidal cells in transgenic mice expressing chimeric NMDAR GluN2 subunits

Authors: *R. KEITH[1], J. M. AZCARATE[2], T. C. DUMAS[1], Z. SAFI[2], M. F. BADAKHSH[2], M. J. KEITH[3], K. S. ZECHMAN[4], G. J. LEE[4];
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Abstract: Experience-dependent activation of N-methyl-D-aspartate receptors (NMDARs) at hippocampal excitatory synapses initiates structural changes that support memory formation. These structural changes include greater dendritic arbor complexity and increased spine number. Two predominant signaling properties of NMDARs that have been independently linked to hippocampal plasticity are calcium conductance into the postsynaptic spine and direct intracellular protein interactions. These properties vary with the composition of the NMDAR such that, compared to NMDARs with GluN2A subunits, NMDARs with GluN2B subunits conduct calcium for a longer period after activation and display greater affinity for the obligatory synaptic plasticity protein, CaMKII. As such, it is not possible to determine the separate influences of these NMDAR properties by switching the entire GluN2 subunit. To overcome this obstacle, we constructed GluN2 chimeras and expressed them in transgenic mice. Conductance regulating domains exist in the GluN2 extracellular amino (A)-terminus and transmembrane (TM) regions. Intracellular signaling domains exist in the intracellular carboxy (C) terminus. We generated two transgenic mouse lines, one having the A-terminus and TM regions of GluN2A fused to the C-terminus of GluN2B (termed ABc) and, vice versa, the other line having the A-terminus and TM regions of GluN2B fused to the C-terminus of GluN2A (termed BAc). These chimeric GluN2 subunits were expressed using the tet-off expression system with tetracycline transactivator protein (tTA) expression under transcriptional control of the CaMKII minimal promoter. tTA expression was seen in many forebrain regions, but predominantly in hippocampal pyramidal cells. To examine how separate NMDAR properties regulate dendritic structure, we measured neuromorphological characteristics of hippocampal pyramidal neurons in young adult mice (1-2 months of age), utilizing both Golgi-Cox staining and Thy-1 GFP

fluorescence methods. Bright field and confocal microscopy, along with Neurolucida tracing, were employed to measure dendritic arbor complexity and spine density. Preliminary results demonstrate a trend for reduction in dendritic branching and spine density in ABc compared to BAc and wild-type (WT) animals. Given the heavy GluN2A background in adult mice, these results suggest potentially stalled development due to greater presence of the GluN2B C-terminus. These findings advance understanding of the means by which NMDARs regulate dendritic plasticity/development by better defining the region of the GluN2B subunit that restrains hippocampal development.

Disclosures: **R. Keith:** None. **J.M. Azcarate:** None. **T.C. Dumas:** None. **Z. Safi:** None. **M.F. Badakhsh:** None. **M.J. Keith:** None. **K.S. Zechman:** None. **G.J. Lee:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.03/D32

Topic: B.02. Ligand-Gated Ion Channels

Title: Exploration of the molecular mechanisms underlying the effect of L-lactate on long term memory

Authors: E. K. IBRAHIM[1], O. AL ZAHRANI[1], H. FIUMELLI[1], *P. J. MAGISTRETTI[2,1]; [1]Biol. and Envrn. Sci. and Engin., King Abdullah Univ. for Sci. and Technol., Thuwal, Saudi Arabia; [2]Ecole Polytechnique, Lausanne, Switzerland

Abstract: In the brain, glycogen, the storage form of glucose, is exclusively localized in astrocytes (Magistretti and Allaman, 2015). Glycogenolysis leads to the production of L-lactate, which is shuttled to neurons for ATP production. Interestingly, L-lactate was recently shown to be not only a source of energy, but also a signaling molecule to neurons. This was demonstrated through the inhibition of L-lactate production or transport in an inhibitory avoidance paradigm, where the rodents developed amnesia. This inhibition of memory consolidation was rescued by L-lactate and not by equicaloric glucose emphasizing that L-lactate acts as a signaling molecule as well (Suzuki et al., 2011). A recent study in our laboratory suggests that the action of L-lactate takes place through a cascade of molecular events via the modulation of N-methyl-D-aspartate receptor (NMDAR) activity (Yang et al., 2014). Since NADH produced similar results to those seen with L-lactate, it was hypothesized that the action of the latter is based on altering the redox state of the cell, in particular in view of the fact that redox-sensitive sites are present on the NMDAR. However, the precise molecular mechanism underlying the apparent change in the NMDAR activity is not fully elucidated. The objective of this study is to explore those

mechanisms.
It has been previously shown that when key cysteines on NMDARs are reduced, the activation of NMDAR is amplified and vice versa (Choi et al., 2001). Accordingly, we introduced point mutations at those cysteines on different subunits of NMDAR and expressed them in HEK cells. Fluorescence microscopy for calcium imaging was used to monitor NMDARs activity. Initial results show three main effects of L-lactate on wild type NMDAR composed of the subunits NR1/NR2A. First, faster kinetics of the initiation of response compared to control. Second, L-lactate increased the percentage of cells having a maximum amplitude value greater than 7.5 fold of the baseline calcium signal as compared to the control. Finally, L-lactate significantly decreased the decay rate of the calcium signal to the basal calcium levels of the cells during the washout. In addition, using the mutated NR2A subunit (C87S, C320S) led to changes in the amplitude of the NMDAR responses to glutamate. Thus we have now established a system where the effect of L-lactate can be tested on NMDAR subunits mutated at different redox-sensitive sites.
In conclusion, these results confirm in transfected HEK cells that L-lactate has potentiating effects on NMDAR activity, possibly through the alteration of the redox state of the cell.

Disclosures: E.K. Ibrahim: None. **O. Al Zahrani:** None. **H. Fiumelli:** None. **P.J. Magistretti:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

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Program#/Poster#: 396.04/D33

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH_5R01DA020140 to K.S.J.

Saudi Arabian Cultural Mission (SACM)

Title: Optophysiological characterization of endogenous and recombinant glutamate receptors in neuroblastoma cells

Authors: *N. A. ALMUZAINI[1], K. S. JONES[2];
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Abstract: Optophysiology is an emerging physiological technique that uses genetically encoded sensors such as GCaMP6 to visualize neuronal activity *in vivo* and *in vitro*. Optophysiology may present several advantages over traditional electrophysiological methods and in this study we set

out to determine the feasibility of using the optical calcium sensor, GCaMP6s, to monitor the functional expression of *N*-Methyl-D-aspartate receptors (NMDARs). N-methyl-D-aspartate receptors (NMDARs) are one of three major ionotropic glutamate receptors that mediate excitatory transmission in the central nervous system. To demonstrate the feasibility of our approach we utilized the neuroblastoma cell line, SK-N-SH. Undifferentiated SK-N-SH cells expresses very low levels of NMDA receptor subunits. However, treatment with retinoic acid (RA) has been shown to confer a neuron-like morphology in SK-N-SH cells and increase the expression of NMDA receptors (Pizzi et al, 2002). We hypothesized retinoic acid-treatment would induce sufficient expression of functional NMDARs to permit optophysiological detection and characterization by GCaMP6s in SK-N-SH cell. To test this hypothesis, SK-N-SH cells were cultured in 30 uM RA for 14d. After 14d, the cells were transfected with a plasmid encoding GCaMP6s. Calcium imaging was performed two days later on a spinning-disk confocal microscope. Calcium signals were evoked from SK-N-SH cells by bath applying the NMDAR co-agonist 100 uM NMDA and 100 uM D-Serine. Agonist-dependent calcium signals were detected from RA-treated cells, but not vehicle-treated cells. Moreover, the calcium signals were abolished by co-application of NMDA receptor antagonists, MK-801 and ketamine. We also examined the impact of RA on the morphology of SK-N-SH cells. We found that RA-treatment lowered the density of proliferating cells, increased average neurite length, and increased staining for neuron specific markers such as Tuj1, MAP2, and GluN1/GluN2B receptors. These data demonstrate the feasibility of using GCaMP to physiologically characterize a calcium-permeable, ligand-gated ion channel and confirm the ability of RA to induce a neuron-like morphology in SK-N-SH cells.

Disclosures: **N.A. Almuzaini:** None. **K.S. Jones:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.05/D34

Topic: B.02. Ligand-Gated Ion Channels

Support: Eli Lilly LIFA Fellowship

Title: Epilepsy-associated GRIN2A mutations - functional analysis and pharmacological rescue of phenotypic deficits

Authors: ***L. ADDIS**[1], J. K. VIRDEE[2], L. R. VIDLER[2], D. A. COLLIER[2], D. K. PAL[1], D. URSU[2];

[1]Dept. of Basic and Clin. Neurosci., King's Col. London, London, United Kingdom; [2]Neurosci. Discovery, Eli Lilly Res. Ctr., Windlesham, United Kingdom

Abstract: Epileptic encephalopathies are severe brain disorders characterized by seizures and abundant epileptiform activity contributing to cognitive, language and behavioural regression. An electro-clinical spectrum of epileptic, cognitive and language disorders can be formed from the frequent focal epilepsy Rolandic epilepsy, to the severe epileptic encephalopathies Landau-Kleffner syndrome (LKS) and continuous spikes and waves during slow-wave sleep (CSWS). Around 20% of cases in this spectrum are caused by mutations in the NMDA receptor *GRIN2A*. Here we analyse the disease mechanisms of ten human missense *GRIN2A* mutations and investigate strategies for restoration of functional deficits observed in our study. Human *GRIN2A* mutation constructs were transiently transfected into HEK-293 cells along with human *GRIN1* to form heterotetrameric receptors. Confocal imaging of immunolabelled cells showed normal membrane expression for wild type (WT) and some mutant constructs. However protein from two NR2A mutants were trapped in the endoplasmic reticulum with no surface expression, and another three mutants had vastly reduced levels of membrane expression compared to WT. Western blotting revealed mutations located before the C-terminal domain of NR2A had reduced total expression compared to WT, with those that disrupt the disulphide-bond of cysteine residues expressing less than 50% of WT protein levels. Single cell calcium imaging and patch clamp recordings showed that mutations both at the interface of NR1 and NR2A and close to the glutamate binding site had a robust effect on glutamate affinity, causing a rightward shift of 4 to 30-fold in the concentration dependence for glutamate. These mutations also decreased the affinity of the NMDA receptor to glycine. Mutations located after the glutamate binding domain responded as wild-type to glutamate and glycine. Single cell patch clamping also revealed alterations in the Mg^{2+} block of some mutants. High-throughput calcium flux assays showed that all studied mutations do not appear to alter NMDA receptor antagonist pharmacology. We were able to rescue the phenotype of the mutations with reduced glutamate affinity after treatment with an NR2A-selective positive allosteric modulator. Taken together these data suggest that mutations across *GRIN2A* affect the expression and function of the receptor in different ways, with the end result of altered NMDA receptor currents and neuronal excitability. Careful molecular profiling of these patients is essential for effective personalised treatment options.

Disclosures: **L. Addis:** 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; Contractor for Eli LIlly. **J.K. Virdee:** A. Employment/Salary (full or part-time): Contractor for Eli Lilly. **L.R. Vidler:** A. Employment/Salary (full or part-time): Employed by Eli Lilly. **D.A. Collier:** A. Employment/Salary (full or part-time): Employed by Eli Lilly. **D.K. Pal:** F. Consulting Fees (e.g., advisory boards); Amplexa Genetics. **D. Ursu:** A. Employment/Salary (full or part-time): Employee of Eli Lilly.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.06/E1

Topic: B.02. Ligand-Gated Ion Channels

Support: Russian Scientific Foundation grant 16-15-10192

Title: Peculiarities of agonist activity of homocysteine on GluN2A- and GluN2B-containing NMDA receptors

Authors: *S. M. ANTONOV[1], D. A. SIBAROV[1], P. A. ABUSHIK[1], R. GINIATULLIN[2]; [1]Sechenov Inst. of Evolutionary Physiol. and Biochem., Saint-Petersburg, Russian Federation; [2]Dept. of Neurobio., A. I. Virtanen Inst. for Mol. Sciences, Univ. of Eastern Finland, Kuopio, Russian Federation

Abstract: Hyperhomocysteinemia, a condition with excessive level of homocysteine (HCY) in plasma, leads to neuronal death and underlies a variety of cardiovascular and neurodegenerative disorders. Whereas it is known that HCY can interact with NMDA receptors (NMDARs), its action on receptors composed by different subunit composition is almost unknown. Therefore, using cultured cortical neurons and heterologous expression in HEK293T cells we tested the agonist activity of HCY on GluN1/2A or GluN1/2B subunit containing NMDAR. In neurons responses induced by 50 μM HCY, in contrast to current induced by 30 μM NMDA (both in the presence of 30 μM Gly) were often comparable in amplitude but much faster declined to the baseline. In calcium-free external solution the decrease of NMDA evoked currents was abolished, suggesting the Ca^{2+}-dependence NMDAR desensitization. In contrast, in calcium-free conditions HCY evoked currents still declined almost to the baseline suggesting calcium-independent desensitization. In HEK293T cells HCY activated NMDARs of GluN1/2A and GluN1/2B subunit compositions with EC_{50}s of 9.7 ± 1.8 μM and 61.8 ± 8.9 μM, respectively. Recombinant GluN1/2A receptors, however, did not desensitize by HCY, whereas GluN1/2B receptors were almost fully desensitized by HCY. Thus, HCY is a high affinity agonist of NMDARs preferring the GluN1/2A subunit composition. Our data suggest that HCY induced native NMDAR currents in neurons are mainly mediated by the 'synaptic type' GluN1/2A NMDARs. This implies that in hyperhomocysteinemia HCY may contribute to postsynaptic responses along with excitotoxicity via GluN2B-containing extrasynaptic receptors.

Disclosures: S.M. Antonov: None. **D.A. Sibarov:** None. **P.A. Abushik:** None. **R. Giniatullin:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

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Program#/Poster#: 396.07/E2

Topic: B.02. Ligand-Gated Ion Channels

Support: VMCVM/VCOM Center for One Health Seed Grant proposal 4-59032

Title: Positive modulatory interactions of NMDA receptor GluN1/2 ligand binding domain attenuate competitive antagonists activity

Authors: D. BLEDSOE[1], C. TAMER[2], I. MESIC[2], C. MADRY[3], H. BETZ[4], *B. G. KLEIN[5], B. LAUBE[2], B. COSTA[6];
[1]Biochem., Virginia Tech., Blacksburg, VA; [2]Technische Univ. Darmstadt, Darmstadt, Germany; [3]Univ. Col. London, London, United Kingdom; [4]Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany; [5]Biomed. Sci. and Pathobiology, Virginia Tech, Col. of Vet. Med., Blacksburg, VA; [6]Biochem. and Pharmacol., Edward Via Virginia Col. of Osteo. Med., Blacksburg, VA

Abstract: The N-methyl D-aspartate (NMDA) receptors play a crucial role in normal brain function and pathogenesis of neurodegenerative and psychiatric disorders. Functional tetraheteromeric NMDAR contains two obligatory GluN1 subunits and two identical or different non-GluN1 subunits that include six different gene products; four GluN2 (A-D) and two GluN3 (A-B) subunits. The heterogeneity of the subunit combination determines the distinct function of NMDARs. All GluN subunits contain an extracellular N-terminal Domain (NTD) and ligand binding domain (LBD), a transmembrane domain (TMD) and an intracellular c-terminal domain (CTD). The interaction between the GluN1 and coassembling GluN2 or N3 subunits through the LBD has been proven crucial for defining receptor deactivation mechanisms that are unique for each combination of NMDAR. Modulating the LBD interactions has great therapeutic potential. In the present work, by amino acid point mutations and two electrode voltage clamp electrophysiology techniques, we have studied the role of LBD interactions in determining the effect of well characterized pharmacological agents including agonists, competitive antagonists, uncompetitive antagonists and allosteric modulators. The results reveal that the LBD interaction between GluN1&2 through GluN1 Y535 and E781 plays a decisive role in determining affinity and efficacy of competitive, but not noncompetitive, antagonists. These findings revitalize the modulatory role of LBD interactions that can be exploited to design and develop novel NMDAR based therapeutic agents.

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Poster

396. NMDA Receptors II

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Program#/Poster#: 396.08/E3

Topic: B.02. Ligand-Gated Ion Channels

Support: Canadian Institutes of Health Research MOP-12699

Canadian Institutes of Health Research FDN 143210

Title: Regulation of striatal neuronal NMDAR trafficking by palmitoylation: Potential role in Huntington disease

Authors: *R. KANG[1], L. WANG[2], S. S. SANDERS[3], K. ZUO[2], M. R. HAYDEN[3], L. A. RAYMOND[2];
[2]Dept. of Psychiatry and Djavad Mowafaghian Ctr. for Brain Hlth., [3]Dept. of Med. Genet. and Ctr. for Mol. Med. and Therapeut., [1]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: N-methyl-D-aspartate receptors (NMDAR) play a critical role in excitatory synaptic signaling, and alterations in the balance of synaptic and extrasynaptic NMDARs impact neuronal survival. Recent studies show enhanced extrasynaptic GluN2B-type NMDAR activity in striatal neurons in the YAC128 mouse model of Huntington disease (HD), resulting in increased and decreased activation of cell death and survival pathways, respectively, that contribute to striatal vulnerability to degeneration in HD. Mechanism(s) of altered GluN2B trafficking remain unclear, however. Notably, the huntingtin (Htt) protein directly interacts with palmitoyl acyltransferases DHHC17 and DHHC13, also called huntingtin-interacting protein-14 (HIP14) and HIP14-like (HIP14L) and mutant Htt expression results in reduced interaction with HIP14 and HIP14L, leading to decreased palmitoylation of several synaptic proteins in YAC128 mice. Furthermore, GluN2B palmitoylation on two C-terminal cysteine clusters regulates its trafficking to surface membrane and synapses in cortical neurons. Here, we investigated whether altered GluN2B palmitoylation contributes to its accumulation at extrasynaptic sites in striatal neurons from YAC128 HD mice. We found reduced GluN2B palmitoylation in YAC128 striatum. Moreover, NMDAR containing the cluster II (but not cluster I) palmitoylation-resistant mutant GluN2B (GluN2B 5CS) showed significantly enhanced surface expression in striatal neurons in wild-type (FVB/N) corticostriatal co-cultures, mimicking the increased striatal GluN2B surface expression observed in YAC128 co-cultures. Importantly, the increased striatal surface GluN2B 5CS was restricted to extrasynaptic membranes. Furthermore, we found HIP14 and HIP14L differentially interact with and palmitoylate GluN2B on the two clusters *in vitro*. These findings suggest potential roles of HIP14 and HIP14L in altered GluN2B-NMDAR trafficking in striatal neurons of an HD mouse model.

Disclosures: **R. Kang:** None. **L. Wang:** None. **S.S. Sanders:** None. **K. Zuo:** None. **M.R. Hayden:** None. **L.A. Raymond:** None.

Poster

396. NMDA Receptors II

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Program#/Poster#: 396.09/E4

Topic: B.02. Ligand-Gated Ion Channels

Support: K12 NS049453

R21 NS081439-02

Title: Human anti-GluN1 antibody-mediated effects on NMDA receptor subtypes.

Authors: ***J. A. PANZER**, A. RATTELLE, D. R. LYNCH;
Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are heterotetrameric glutamate receptors composed of two GluN1 subunits, and two other subunits, most often GluN2A or GluN2B. Neuronal NMDARs are located at both synaptic and extrasynaptic locations; signaling from receptors at synaptic sites can promote synaptic plasticity and cell survival whereas extrasynaptic signaling has been linked to neurodegeneration. GluN2A-containing receptors may be more prevalent at synaptic sites, while GluN2B containing receptors are often associated with extrasynaptic sites. In the autoimmune disease anti-NMDAR encephalitis, antibodies bind the GluN1 subunit of the NMDAR, resulting in psychosis, altered consciousness, seizures, dyskinesias, and autonomic dysfunction. Antibodies bind to the GluN1 extracellular amino-terminal domain, resulting in transient stabilization of the receptor's open conformation, and subsequent NMDAR hypofunction due to receptor cross-linking and internalization. We have found that the impact of patients' anti-GluN1 antibodies on NMDARs is modulated by the GluN2 subtype, resulting in distinct action on GluN2A versus GluN2B containing receptors. We have further evaluated the effects of antibody binding on neuronal activity, synaptic versus extrasynaptic NMDARs, and downstream signaling. Our results may explain why the clinical symptoms of the disease do not precisely replicate global NMDAR knockdown, and may point towards potential therapeutic targets in anti-NMDAR encephalitis. The unique properties of these antibodies also raise the possibility of their use as tools to explore NMDAR function.

Disclosures: **J.A. Panzer:** None. **A. Rattelle:** None. **D.R. Lynch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent for testing anti-NMDAR antibodies with royalties paid to Eurimmune.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.10/E5

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NS 060756

NSF Graduate Research Fellowship Program

Title: Lateral mobility of synaptic NMDA receptors in hippocampal slices

Authors: ***A. L. MCQUATE**, A. BARRIA;
Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: NMDARs are fundamental coincidence detectors necessary for the induction of synaptic plasticity. Adjusting NMDAR synaptic content, whether by receptor insertion via intracellular vesicles or lateral diffusion between extrasynaptic and synaptic compartments, could play a substantial role defining the characteristics of the NMDAR-mediated EPSC, which in turn would mediate the ability of the synapse to undergo plasticity. Electrophysiological evidence from cultured autapses and fluorescence imaging of single molecules in dissociated hippocampal neurons both suggest that NMDARs diffuse laterally between extrasynaptic and synaptic compartments. However, attempts to demonstrate this motility in hippocampal slices thus far have failed. To test for lateral mobility in hippocampal slices, we rapidly blocked synaptic NMDARs using MK-801. Following a five-minute washout period, we recovered roughly 50% of baseline NMDAR EPSC amplitudes. The degree of the observed recovery was proportional to the amount of induced blockade. These results indicate that the lateral diffusion of NMDARs could be a mechanism by which synapses rapidly adjust parameters to fine tune synaptic plasticity.

Disclosures: **A.L. McQuate:** None. **A. Barria:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.11/E6

Topic: B.02. Ligand-Gated Ion Channels

Title: Characterisation of two NMDA NR2B subunit (grin2b) antagonists across tests of impulsivity and attention

Authors: *G. A. HIGGINS[1,2], L. B. SILENIEKS[1], C. MACMILLAN[3], J. SEVO[3], F. D. ZEEB[4,2], S. THEVARKUNNEL[1];
[1]Intervivo Solutions Inc, Toronto, ON, Canada; [2]U. Toronto, Toronto, ON, Canada; [3]Vivocore, Toronto, ON, Canada; [4]CAMH, Toronto, ON, Canada

Abstract: NMDA NR2B subtype selective antagonists are currently in clinical development for a variety of indications, including major depression. We previously reported the selective NR2B NMDA antagonists Ro 63-1908 (Ro) and traxoprodil, increased premature responding in a rat 5-choice serial reaction time task (5-CSRTT) suggesting an effect on impulsive action (Higgins et al (2005) Psychopharmacology 179: 85-98). The present studies extend these investigations to a Go-NoGo and delay discounting task, and the 5-CSRTT under test conditions of both regular (5s) and short (2-5s) multiple ITI. Dizocilpine was included for comparison. Male Long-Evans rats were used in all experiments. Both Ro 63-1908 (0.1-1mg/kg SC) and traxoprodil (0.3-3mg/kg SC) increased premature and perseverative responses in both 5-CSRT tasks and improved attention when tested under a short ITI test condition (e.g. premature: vehicle: 11.8$\pm$2.6 responses, Ro 1 mg/kg: 68.3$\pm$17.6 responses; $P<0.01$). Ro 63-1908 but not traxoprodil increased motor impulsivity (false alarms) in a Go-NoGo task (vehicle: 8.1$\pm$1.5 responses, Ro 1 mg/kg: 14.3$\pm$2.5 responses; $P=0.02$). Dizocilpine (0.01-0.06mg/kg SC) affected both measures of motor impulsivity and marginally improved attention. In an ascending delay discounting test of impulsive choice, both dizocilpine and Ro 63-1908 decreased impulsive choice (i.e. increased choice for the larger, delayed reward), while traxoprodil showed a similar trend (e.g. AUC measure: vehicle: 1006$\pm$167 units, Ro 1 mg/kg: 1358$\pm$199 units; $P<0.01$). Motor stimulant effects were evident following Ro 63-1908 (0.3-3 mg/kg SC), but not traxoprodil (1-10 mg/kg SC) treatment - although no signs of motor stereotypy characteristic of dizocilpine dose (>0.1 mg/kg) were noted. The findings of both NR2B NMDA antagonists affecting measures of impulsive action and compulsive behavior may underpin emerging evidence to suggest glutamate signaling through the NMDA NR2B receptor plays an important role in behavioural flexibility (e.g. Holmes et al (2013) Nature Neurosci. 16: 1101-1110.). The profiles between Ro 63-1908 and traxoprodil were not identical, perhaps suggesting differences between members of this drug class on behaviour.

Disclosures: **G.A. Higgins:** None. **L.B. Silenieks:** None. **C. MacMillan:** None. **J. Sevo:** None. **F.D. Zeeb:** None. **S. Thevarkunnel:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.12/E7

Topic: B.02. Ligand-Gated Ion Channels

Support: Grant from Innovation Fund Denmark

Title: N-Methyl-D-Aspartate (NMDA) receptor glycine site agonist shows pronounced subtype-dependent pharmacological profiles

Authors: ***M. JESSEN**[1,3], K. FREDERIKSEN[1], H. BRÄUNER-OSBORNE[3], P. KILBURN[2], A. DAMHOLT[1];
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Abstract: *N*-methyl-D-aspartate (NMDA) receptors are tetrameric ligand-gated ion channels involved in memory function as well as neurological diseases, including depression and Parkinson's disease. There are several small-molecule binding sites on the different NMDA receptor subtypes and understanding of the pharmacology of these receptors can facilitate the development of new therapeutic agents. A single study has shown that the glycine site partial agonist D-cycloserine (DCS) has antidepressive effects in clinic. Subtype-dependent differences in agonist efficacy have previously been reported for DCS. Using two-electrode voltage-clamp electrophysiology, we have characterized compound I, a potent glycine site agonist, at four different human NMDA receptor subtypes (GluN1/2A-2D) expressed in *Xenopus* oocytes. Compound I showed pronounced subtype-dependent pharmacological activity, and was a full agonist at GluN1/2A (I_{max} = 100% relative to glycine, EC_{50} = 66 nM), a super agonist at GluN1/2C (I_{max} = 476%, EC_{50} = 8 nM), and was a partial agonist at GluN1/2B and GluN1/2D (I_{max} = 10% and 58%, EC_{50} = 14 nM and 33 nM respectively). This is intriguing, since compound I binds the glycine site of the GluN1 subunit, which is conserved in all four heteromeric receptors. To delineate the mode-of-action, compound I was tested on GluN1/2A receptors with three single point mutations (F484A, R523A, and T518L) in the glycine binding site. These three mutations greatly reduced compound I potency by 400- to 1100-fold at the four NMDA receptor subtypes without changing agonist efficacy relative to glycine, indicating that compound I binds in the GluN1 glycine binding pocket. The striking functional GluN1/2C selectivity of compound

I is unprecedented among any glycine site agonists evaluated, including DCS. Compound I is therefore an interesting tool compound for uncovering the mechanism of glycine site-mediated GluN2-dependent subtype selectivity and for future drug design.

Disclosures: **M. Jessen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **K. Frederiksen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **H. Bräuner-Osborne:** None. **P. Kilburn:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **A. Damholt:** A. Employment/Salary (full or part-time): H. Lundbeck A/S.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.13/E8

Topic: B.02. Ligand-Gated Ion Channels

Support: NICHD Intramural Award to C.J.M

NINDS Competitive Fellowship Award to J.C.W

Title: Investigating the impact of NMDA receptor hypounction on the synaptic integration of hippocampal neurogliaform cells

Authors: *R. CHITTAJALLU, J. C. WESTER, M. C. CRAIG, E. BARKSDALE, G. AKGUL, S. HUNT, C. FANG, X. YUAN, D. COLLINS, K. A. PELKEY, C. J. MCBAIN;
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Abstract: Neural network dysfunction can result from excitation and inhibition imbalance, the latter being mediated by diverse interneuron (IN) subtypes each endowed with unique functional properties. Appropriate specification and synaptic incorporation into neural circuits of IN subtypes is critical for normal CNS function. Although perisomatic inhibition of principal cells, such as that imparted by parvalbumin-expressing (PV) basket cells, shapes network behavior the importance of dendritic inhibition in controlling excitatory output, generating oscillations and gating information flow is gaining more attention. Neurogliaform cells (NGFCs) are the prevalent family of dendritic targeting INs within the hippocampus yet one of the most understudied. NMDAR activity is central in many developmental and synaptic processes and their hypofunction, particularly in PV basket cells, is implicated to be causal in disorders such as schizophrenia and autism. Here, we show that NGFCs in the CA1 stratum lacanosum-moleculare (SLM) possess large synaptic NMDAR-mediated responses throughout postnatal development (~10-fold higher NMDAR/AMPAR ratio than in PV basket cells). We therefore examined the

relevance of NMDARs in NGFC development. To ablate NGFC NMDARs we crossed floxed *Grin1* knockout and *Htr3A*-cre:Ai14 mouse lines. NMDAR hypofunction resulted in lower density of TdTom+ cells in SLM and reduction of reelin expression, a marker for NGFCs, in the remaining tdTom+ cells. Functionally, although NMDAR knockout in NGFCs did not alter basic membrane and firing properties, an increase in AMPAR-mediated sEPSC frequency that progressively worsened during postnatal development was evident. The normal developmental trajectories of AMPAR-mediated sEPSC amplitude and paired pulse ratios were significantly retarded. Changes in AMPAR inward rectification suggestive of GluA2 loss was also observed and a marked misspecification of dendritic arborization was noted. These data demonstrate NMDAR hypofunction precipitates pre- and post-synaptic abnormalities in NGFCs. The predominant glutamatergic afferents impinging on NGFCs arise from two brain regions - layer III of medial entorhinal cortex and thalamic nucleus of reuniens. Using optogenetics, we are currently assaying whether the observed synaptic changes are afferent specific and hence may impact relative information processing between these brain structures and hippocampus. Future studies will be required to decipher the pathophysiological consequences of NMDAR hypofunction in NGFCs thus leading to further insight into the underlying molecular and synaptic etiology of neurodevelopmental disorders.

Disclosures: R. Chittajallu: None. **J.C. Wester:** None. **M.C. Craig:** None. **E. Barksdale:** None. **G. Akgul:** None. **S. Hunt:** None. **C. Fang:** None. **X. Yuan:** None. **D. Collins:** None. **K.A. Pelkey:** None. **C.J. McBain:** None.

Poster

396. NMDA Receptors II

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Support: NIMH Grant MH082304

NIH Neuroscience Training Program in Neurobiology of Cognition and Cognitive Disorders 5T32NS061788-08

Howard Hughes Medical Institute Med-Grad Program

Title: Low-dose NMDAR antagonists increase the excitation/inhibition balance onto CA1 pyramidal cells causing disinhibition

Authors: *A. J. WIDMAN, L. L. MCMAHON;
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Abstract: Low-dose NMDAR antagonists, including ketamine, elicit rapid antidepressant responses in patients with Major Depressive Disorder (MDD) and in preclinical rodent models, although the anti-depressant efficacy is variable between mechanistically distinct NMDAR antagonists. Blocking NMDARs on pyramidal cells or on parvalbumin basket cells are proposed mechanisms to trigger the antidepressant effects, but the location of relevant NMDARs remains unclear. To date, no study has examined the most immediate effects of NMDAR inhibition on the excitation/inhibition (E/I) balance in hippocampus, a brain region negatively impacted by depression. To investigate whether NMDAR inhibition causes a net increase in excitation, we examined the effect of NMDAR antagonists on spontaneous IPSCs and EPSCs, evoked E/I ratio, and synaptically driven action potentials in hippocampal pyramidal cells in acute slices. Additionally, we tested whether three mechanistically different NMDAR antagonists: ketamine, a non-competitive antagonist, GLYX-13, a partial antagonist, and Ro 25-6981, a GluN2B subunit selective antagonist, all of which have antidepressant effects at low-dose, produce shared or distinct effects. Ketamine decreased both sIPSC frequency and amplitude, without altering either sEPSC frequency or amplitude, implicating a selective effect on inhibition. The E/I ratio increased following application of ketamine. Also, we found an increased probability that evoked subthreshold EPSPs will generate a synaptically driven action potential in the presence of ketamine, showing that pyramidal cells are disinhibited. GLYX-13 reduces spontaneous and evoked inhibition to a lesser degree and produces a less potent disinhibition compared to ketamine, which is likely explained by its partial agonist activity. Ro 25-6981 elicited a variable effect with sIPSC frequency increasing in some cells and decreasing in others. This was also observed with the E/I ratio and synaptically driven action potential probability. Ro 25-6981 increased inhibition in some cells and increased excitation in others, which may be due to a subset of interneurons having GluN2B-containing NMDARs. Together, our results show that ketamine and GLYX-13 shift the E/I balance towards excitation and disinhibit pyramidal cells, likely through targeting NMDARs on interneurons. Results also demonstrate that Ro 25-6981 does not increase the E/I balance suggesting mechanistically distinct NMDAR antagonists have different effects on synaptic circuit dynamics potentially providing a mechanistic understanding of the lower efficacy of GluN2B-selective NMDAR antagonists in treating depression.

Disclosures: A.J. Widman: None. **L.L. McMahon:** None.

Poster

396. NMDA Receptors II

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Title: Structure activity relationships and mechanism of action of NMDA receptor positive allosteric modulators based upon 2-naphthoic acid

Authors: *K. SAPKOTA[1], G. FANG[2], M. W. IRVINE[2], E. BURNELL[2], G. CULLEY[2], D. CHOPRA[3], S. DRAVID[3], G. L. COLLINGRIDGE[4], D. E. JANE[2], D. T. MONAGHAN[1]; [1]Dept. of Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE; [2]Ctr. for Synaptic Plasticity, Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [3]Dept. of Pharmacol., Creighton Univ., Omaha, NE; [4]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: N-Methyl-D-aspartate (NMDA) receptor hypofunction is thought to significantly contribute to the symptoms of schizophrenia and, perhaps, autism. Thus, NMDA receptor positive allosteric modulators are potential therapeutics for the treatment of these conditions. Our laboratories have previously reported the properties of NMDAR PAMs based upon the phenanthroic acid with an alkyl chain. More recently, we have evaluated a family of related 6-alkyl-2-naphthoic acid analogues and find several highly effective NMDAR PAMs with optimal activity corresponding to a pentyl chain length with weak activity for propyl and butyl groups and inhibitory activity for an ethyl substituent. Polar substitutions along the alkyl chain generally inhibited PAM activity. Of the compounds identified, UBP684 and UBP753, were among the most effective in enhancing NMDAR responses at the four GluN1/GluN2 receptors (GluN1/GluN2A-GluN2D). The potentiation by these compounds is voltage-independent and is not blocked by saturating concentrations of agonist (L-glutamate plus glycine). Unlike that of a phenanthroic acid PAM (UBP512), UBP684 can bind to the closed state of the receptor. Using GluN1/GluN2A and GluN1/GluN2D receptors, we find that UBP684 slows deactivation time of the receptor. We have also found that when the glutamate binding site is locked in the agonist conformation by a disulfide bridge with two cysteine mutations in GluN2A, the potentiation is greatly diminished. However, locking the GluN1 glycine binding site in the agonist conformation does not reduce potentiation. These results are consistent with single channel analysis indicating that UBP684 potentiates by increasing the open probability and more effectively modulates gating steps controlled by the GluN2 subunit than by the GluN1 subunit. Computer modelling suggests that these compounds can bind in GluN1/GluN2 ligand binding domain (LBD) interface near the hinge of the LBD. In particular, the longer alkyl chain naphthoic acid analogues, which have PAM activity, are long enough to place the end of their alkyl chain near the hinge region of UBP684 in a hydrophobic pocket that would not tolerate polar substituents. By analogy to AMPA receptor PAMs, binding at this location can account for the NMDAR potentiation and slowed receptor deactivation of these modulators. These results suggest a mechanism of action

for the newly characterized NMDAR PAMs and structural insights that may lead to improved lead compounds for neuropsychiatric disorders.

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Poster

396. NMDA Receptors II

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Support: NIH_#R01DA020140 to K.S.J

Title: Protons exert distinct influences on the translocation of the M3 domains of GluN1 and GluN2A N-methyl-D-aspartate receptor subunits

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[1]Biol. Dept., Howard Univ., Washington, DC; [2]Anat., Howard Univ. Col. of Med., Washington, DC

Abstract: N-methyl-D-aspartate receptors (NMDARs) are one of three major ionotropic glutamate receptors that mediate excitatory transmission in the central nervous system. NMDAR current is strongly attenuated by extracellular proton concentration (pH), which can fluctuate dramatically during pathological states. Several amino acid residues in the transmembrane domain have been shown to contribute to the pH sensitivity of NMDA receptors, however, details of the biophysical mechanism remain unclear. The M3 transmembrane domain of NMDAR subunits undergo conformational rearrangement during channel activation, and we and others have used the Substituted Cysteine Accessibility Method (SCAM) to reveal that sulfhydryl specific modification of the NMDA receptors containing a cysteine substitution at the 7th alanine of the SYTANLAAF motif of the M3 domain (A7C) stabilizes the channel in an open state (Jones et al., 2002; Yuan et al., 2005).
NMDARs comprised from GluN1/GluN2B subunits are trapped in a non-conducting state by protons (Banke et al., 2005). In this study, we used NMDARs comprised from GluN1-A7C or GluN2A-A7C subunits to investigate the impact of protons on the conformational rearrangement of the M3 domain during gating. NMDARs current was measured in *Xenopus laevis* oocytes expressing wild-type or A7C mutant NMDAR subunits using two-electrode voltage-clamp electrophysiology. Proton inhibition was not significantly altered in NMDARs comprised from

GluN1-A7C or GluN2A-A7C subunits, but could be dramatically attenuated by co-application of agonists and 2-Aminoethyl methanesulfonate (MTSEA). Curiously, proton block was more rapidly attenuated by MTSEA modification of NMDARs composed from GluN2A-A7C subunit. Moreover, MTSEA modification increased the amplitude of current evoked from NMDARs comprised from GluN2A-A7C-containing receptors at all pH values. By contrast, MTSEA modification increased the amplitude of current evoked from GluN1-A7C-containing receptors only at pH values less than or equal to 7.3, and decreased the amplitude of current evoked at higher pH.
Together these findings suggest protons may impact the conformational rearrangement of NMDA receptor subunits by restricting the translocation of the channel gate. Moreover, these data suggest the gating elements of the GluN1 and GluN2A subunits have distinct sensitivities to pH and may adopt unique conformational states during channel activation.

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Poster

396. NMDA Receptors II

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Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS NS088479

Title: Endogenous expression of SAP97 in PV interneurons is correlated to decreased NMDAR synaptic activity

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Abstract: Parvalbumin (PV) expressing interneurons are the most common subtype of GABAergic inhibitory interneurons in the cortex. PV interneurons play an essential role in cortical sensory processing and working memory and their dysfunction is associated to disease states such as schizophrenia and epilepsy. The inhibitory output of PV interneurons is driven by glutamatergic excitatory inputs, mediated by ionotropic glutamatergic receptors (AMPARs and NMDARs). The regulation and dynamics of glutamatergic receptors, including the number and subunit composition, will therefore have a strong impact on PV interneuron activity. SAP97 is a scaffolding protein capable of interacting with AMPARs and NMDARs, regulating synaptic trafficking and targeting of the receptors in a subtype and subunit specific manner. SAP97 expression is developmentally downregulated in PV interneurons, leading to two adult

subpopulations distinguished by the presence (SAP97+) or absence (SAP97-) of SAP97 (Akgul and Wollmuth, 2010 J Comp Neurol). Functionally, SAP97 expression in PV interneurons is causally associated to an increase in the frequency of AMPAR-mediated miniature EPSCs (mEPSCs) (Akgul and Wollmuth, 2013 J Neurosci). In the present study we address whether SAP97 expression has any effect on synaptic NMDARs. For that purpose, we use brain slice recordings of NMDAR-mediated mEPSCs from PV interneurons in layers 2-3 of primary visual cortex in juvenile and adult mice, combined with single cell RT-PCR to establish the endogenous expression of SAP97. Our results suggest that SAP97 expression has no effect in juvenile; but in the adult, contrary to the AMPARs, the presence of SAP97 was correlated to a decrease in the frequency of NMDAR-mediated mEPSCs. Consequently the two subpopulations of PV interneurons based on SAP97 expression would have a very different functional profiles, with SAP97+ interneurons having the excitatory input dominated by AMPARs, whereas SAP97- interneurons having both AMPAR and NMDAR components.

Disclosures: R.C. Ferrer Fierro: None. **A. Baez:** None. **L. Wollmuth:** None.

Poster

396. NMDA Receptors II

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Title: Presynaptic NMDA receptors rely on RIM1α β to control readily-releasable pool at synapses onto layer-5 pyramidal neurons

Authors: *Y. CHOU[1], T. ABRAHAMSSON[1], S. LI[1], A. MANCINO[1], E. NURO[1], R. P. COSTA[2], K. A. BUCHANAN[3], D. ELGAR[3], A. V. BLACKMAN[3], A. T. JONES[3], K. MURAI[1], P. J. SJÖSTRÖM[1];
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Abstract: At central synapses, presynaptic NMDA receptors (preNMDARs) typically enhance vesicle release, although the molecular mechanisms are largely unknown. Using quadruple whole-cell recordings, we explored the effect of preNMDAR blockade on spontaneous and evoked release onto layer-5 pyramidal cells in P11-17 mouse visual cortex acute slices. During 30-Hz firing, the GluN2B-specific NMDAR blocker Ro25-6981 (Ro) reduced both readily releasable pool (RRP) size (-36% ± 7%, n = 11 vs. controls -7.1% ± 5%, n = 11, $p < 0.05$) and replenishment rate (-31% ± 7% vs. 18% ± 7%, $p < 0.001$). In evoked release, preNMDARs thus indirectly increase release probability by upregulating the RRP replenishment rate, which a computer model showed improves signal to noise during high frequency firing. PreNMDAR blockade only reduced neurotransmission at firing frequencies above ~8.5 Hz, yet spontaneous release at ~2.5 Hz was also downregulated, suggesting independent mechanisms of regulation. To elucidate the mechanism, we tested if preNMDARs rely on the vesicle pre-priming and presynaptic scaffolding protein RIM1. Heterozygous RIM1αβ knockout (hetKO) mice exhibited decreased EPSC amplitude (32 ± 4 pA, n = 36 vs. controls 54 ± 10 pA, n = 22, $p < 0.01$) and increased paired pulse facilitation (-0.30 ± 0.05 vs. controls -0.47 ± 0.03, $p < 0.001$), indicating reduced release probability. During evoked release in RIM1αβ KO slices, preNMDAR blockade did not affect EPSC amplitude (96% ± 4%, n = 12 vs. 98% ± 10%, $p = 0.88$), RRP replenishment rate ($p=0.83$), or RRP size ($p=0.68$), while in RIM1αβ flox controls all three were reduced (52% ± 10%, n = 6, $p < 0.01$; -69% ± 5%, $p < 0.001$; and -42% ± 9%, $p < 0.01$; ANOVAs). Consistent with preNMDARs relying on RIM1, GluN2B also co-immunoprecipated RIM1. However, preNMDAR blockade still reduced spontaneous release rate in both hetKO and control slices (AP5 in hetKO 77% ± 5%, n=9, $p<0.01$ vs. control; AP5 in floxed controls 75% ± 3%, n=7, $p<0.001$; Ro in hetKO 83% ± 3%, n=7, $p<0.01$; Ro in wild type 82% ± 5%, n=10, $p<0.05$). We next explored if preNMDARs need RIM1 for scaffolding or for signaling. If the former, then preNMDAR-mediated bouton Ca^{2+} supralinearities should be absent in RIM1αβ KO mice. We found however, that the magnitude of Ca^{2+} supralinearities were indistinguishable between RIM1αβ KO and control animals (25% ± 4%, n= 26 vs. 26% ± 8%, n= 18, $p = 0.86$). Since preNMDAR-mediated Ca^{2+} supralinearities were unaffected in boutons of RIM1αβ KO animals, we conclude that preNMDARs signal via RIM1 to boost evoked neurotransmission during high frequency firing by indirectly increasing release probability, whereas preNMDARs regulate spontaneous release via an independent mechanism.

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Poster

396. NMDA Receptors II

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NARSAD Distinguished Investigator Grant: 21809

NIH Grant MH103950

Title: Emerging role of GluN2D-containing NMDARs in modulating synaptic plasticity within the bed nucleus of the stria terminalis and anxiety/depressive-like behaviors.

Authors: *G. J. SALIMANDO[1,2,3,4,5], T. A. WILLS[6], D. G. WINDER[1,2,3,4,5];
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Abstract: The bed nucleus of the stria terminalis (BNST) plays an important role in regulating affective stimuli such as stress and anxiety that have been well-established as precipitating factors for major depressive disorder. Our previous studies suggest an important role for GluN2B-containing N-methyl-D-aspartate receptors (NMDARs) in BNST long term potentiation (LTP), and in regulation of affective behaviors. We used a proteomic strategy to analyze BNST tissues and identify GluN2B-associated proteins within the region. One candidate of interest identified by this study was the GluN2D NMDAR subunit. Utilizing RNAscope® fluorescent *in* situ hybridization, we find that GluN2B mRNA is present in ~95% of observed BNST cells expressing corticotropin releasing factor (CRF) mRNA, and that ~70% of these CRF+ cells also express GluN2D mRNA. Because BNST CRF receptor signaling has been repeatedly shown to participate in the regulation of depression-like behaviors, we elected to explore the role of GluN2D-containing NMDARs in BNST function and behavior. We assessed baseline performance of GluN2D knockout (GluN2D-KO) and wildtype littermate mice across a range of behavioral tasks, including the elevated zero maze (EZM), open field (OF), light/dark box (L/D) and forced swim test (FST) to evaluate the subunit's role in regulating depressive phenotypes. We found that GluN2D-KO mice exhibit phenotypic profiles across these tasks consistent with increased anxiety- and depressive-like behaviors, a result that was noticeable under both group-housed and socially isolated conditions. Further, we are using the GluN2D knockout mouse to explore the potential role of GluN2D in BNST synaptic plasticity. Taken together, these data suggest that GluN2D-containing NMDARs may participate in regulating depressive/anxiogenic behavioral outputs.

Disclosures: **G.J. Salimando:** None. **T.A. Wills:** None. **D.G. Winder:** None.

Poster

396. NMDA Receptors II

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH T32 GM08061

NIH 4R00 AG041225

Title: The role of protein phosphatase 1 in regulating NMDA receptor trafficking

Authors: ***A. M. CHIU**[1], T. TEDESCHI[1], A. SANZ-CLEMENTE[2];
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Abstract: N-methyl-D-aspartate receptors (NMDARs), fundamental components of the excitatory neuronal synapse, play key roles in numerous neurological processes including neuroplasticity and synaptic maturation. NMDARs are able to achieve these phenomenon through their ability to activate specific intracellular cascades in response to receptor activation. Which intracellular cascades are activated upon NMDAR activation is, in part, regulated by the subunit composition and localization of the receptor. Thus, a key aspect of NMDAR regulation and function is tight control over trafficking of the receptor.
Posttranslational modification is a crucial component of receptor trafficking. We have previously investigated a key regulatory aspect of NMDAR trafficking - phosphorylation of the GluN2B PDZ-binding domain. Phosphorylation of this site, by casein kinase 2, results in the disruption of GluN2B-containing NMDARs from synaptic scaffolding proteins. This, in turn, allows for the redistribution of NMDARs away from the synapse. More recently, our efforts have turn towards understanding how the PDZ-binding domain of GluN2B is dephosphorylated, as we hypothesize that the PDZ-binding domain must be dephosphorylated for NMDARs to be integrated into the postsynaptic density. Data generated from our lab has identified protein phosphatase 1 (PP1) as the phosphatase responsible for dephosphorylating GluN2B's PDZ-binding domain. Because PP1 is a constitutive phosphatase, we are currently working towards identifying the molecular mechanism governing PP1's ability to target and dephosphorylate GluN2B's PDZ-binding domain, as well as, understanding the ramifications of altering its activity. Understanding how PP1 exerts regulation on this posttranslational modification and its consequence on NMDAR trafficking and localization may be key to understanding how correct physiological NMDAR trafficking occurs and how this is altered in disease states.

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Poster

396. NMDA Receptors II

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH National Institute on Aging Pioneer Award DP1-AG047744-01

Title: Physiological differences between the primary visual cortex and dorsolateral prefrontal cortex in primate

Authors: *S. YANG[1], M. WANG[2], M. ALTMAN[2], L. E. JIN[2], V. GALVIN[2], A. F. T. ARNSTEN[2], J. A. MAZER[2];
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Abstract: NMDA receptors (NMDAR) subserve persistent neuronal firing of Delay cells during working memory in the dorsolateral prefrontal cortex (dlPFC) of nonhuman primates. In contrast, blockade of AMPAR has only subtle effects on dlPFC Delay cell firing, and the permissive depolarization of the synaptic membrane needed for NMDAR actions is carried out by acetylcholine, e.g via stimulation of alpha-7 nicotinic receptors. The relieance of dlPFC Delay cells on NMDAR has direct clinical relevance, as insults to NMDAR synapses in dlPFC can lead to working memory deficits and thought disorders in patients. Unlike the dlPFC, little is known about the modulation in the primary visual cortex (V1) of the primate. The current study directly compared glutamate receptor influences in an association area (dLPFC) to that in a sensory area (V1) by measuring the spatial receptive field (RF) of single neurons in V1 of the awake monkey while iontophoresis was used to deliver minute amounts of charged compounds near the recorded neuron. Changes in V1 responsivity were compared to changes in dlPFC Delay activity (putative working memory-related) in monkeys performing a visual spatial working memory task. In dlPFC, recent study found that Delay cell persistent firing was abolished by local NR2B NMDAR blockade or by systemic ketamine administration. AMPA receptors contributed background depolarization to sustain network firing, while AMPAR blockade had mixed effects on Delay cell firing, decreasing firing in some neurons, having no effect in others, or increasing firing. In V1, however, low doses of the NMDAR antagonist MK801 or the NR2B NMDAR antagonist Ro25-6981 had little effect on V1 neuronal firing, while high doses of MK801 or Ro25-6981 significantly decreased V1 response to visual stimuli. Interestingly, a low dose of the AMPAR antagonist CNQX significantly suppressed V1 neuronal firing, which was very different from its effects on dlPFC Delay activity. These data show that the modulatory circuitry

in V1 is fundamentally different from that in dlPFC, particularly the circuitry responsible for sustaining delay activity in the absence of visual stimulation in Delay neurons. This difference may account for the resilience of primary visual cortex to the effects of cognitive disorders such as Alzheimer's Disease.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 MH045817

Title: Effects on magnesium block and potentiation of non-pore lining residues in NMDA receptor transmembrane domains

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Abstract: NMDA receptors (NMDARs) are ionotropic glutamate receptors typically composed of two GluN1 and two GluN2 (GluN2A-D) subunits. Each GluN1 and GluN2 subunit contains an extracellular amino terminal domain (ATD) and ligand binding domain (LBD), a transmembrane domain (TMD) and an intracellular C-terminal domain (CTD). The TMD includes three membrane-spanning helices (M1, M3, M4), and a pore-lining reentrant loop (M2). All NMDARs composed of GluN1 and GluN2 subunits (GluN1/2A – GluN1/2D receptors) exhibit voltage-dependent channel block by external magnesium (Mg^{2+}); pore-facing asparagine (N) residues in the M2 regions are thought to form the Mg^{2+} binding site within the ion channel. Interestingly, mutation of an M2 residue in GluN1/2B receptors that is predicted to face away from the pore (GluN2B(W607)) results in loss of Mg^{2+} block (Williams et al., 1998). GluN2B(W607) is predicted to be nearby residues on the adjacent GluN1 M3 region, and may participate in intersubunit interactions. The GluN2A and GluN2B M2 and M3 regions are identical in sequence; in the GluN2A subunit, the W homologous to GluN2B(W607) (GluN2A(W606)) similarly faces the M3 region of the adjacent subunit. However, mutation of GluN2A(W606) results in only a moderate weakening of Mg^{2+} block (Williams et al., 1998). We further investigated the disparate effects on Mg^{2+} block of mutations of GluN2B(W607) and the

GluN2A(W606). Using homology modeling and MD simulations, we identified a residue in the GluN1 M3 region (GluN1(M634)) nearby GluN2A(W606) in GluN1/2A receptors and GluN2B(W607) in GluN1/2B receptors. We used whole-cell electrophysiological recordings from transfected tsA201 cells to examine responses of mutant and wildtype (WT) NMDARs to Mg^{2+}. We found that mutation of GluN1(M634) drastically decreases Mg^{2+} block in GluN1/2A receptors, but has little effect on Mg^{2+} block in GluN1/2B receptors. We also found that mutation of GluN2B(W607) causes augmentation of the previously demonstrated ability of Mg^{2+} to potentiate GluN1/2B receptor responses. Further examination of Mg^{2+} interactions with GluN1/2B receptors revealed that removal the ATD eliminated Mg^{2+} potentiation without affecting channel block by Mg^{2+}. These results reveal an asymmetry between GluN1/2A and GluN1/2B receptors in the influence of TMD mutations on Mg^{2+} block, and that mutation of GluN2B(W607) has complex effects on receptor function.

Disclosures: **M. Wilcox:** None. **S. Mesbahi:** None. **M. Kurnikova:** None. **J.W. Johnson:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.23/E18

Topic: B.02. Ligand-Gated Ion Channels

Support: RFBR Grant 15-04-08283

RFBR Grant 16-04-00653

RFBR Grant 14-04-00227

RSF grant 16-05-10192

Title: Effects of lithium on NMDAR currents and intracellular calcium responses of rat cortical neurons

Authors: *D. A. SIBAROV, E. E. POGUZHELSKAYA, P. A. ABUSHIK, S. M. ANTONOV; Comparative Neurosci., Iephb RAS, Sankt-Peterburg, Russian Federation

Abstract: Lithium treatment is widely used in therapy of affective episodes in schizophrenia and bipolar disorder. However the complex mechanisms of lithium effects are still poorly understood. Previously we have shown that the substitution of lithium for sodium in the external solution reduces whole-cell neuronal currents mediated by N-Methyl-D-Aspartate receptors

(NMDARs), suggesting an acceleration of calcium-dependent NMDARs desensitization. In this study we analyze the relationship between the inhibition of sodium/calcium-exchanger by lithium and the value of calcium-dependent desensitization of NMDARs using the whole cell patch-clamp recordings and calcium imaging (Fluo-3AM) on rat cortical neurons *in vitro*. The partial substitution of lithium for extracellular sodium inhibited NMDA evoked steady-state currents in concentration-dependent manner. The most pronounced inhibition of currents was achieved during full substitution of lithium for sodium which reached the amplitude loss of about 75%. The IC_{50} for lithium effect was found to be of 25-30% of lithium substitution for sodium. The full substitution of lithium for sodium also decreased NMDA evoked calcium responses in neurons. Most likely the lithium effects on NMDARs are caused by an enforcement of calcium-dependent desensitization by the substrate inhibition of the sodium-calcium exchanger, that uncovers the sodium-calcium exchanger function in calcium clearance in the close proximity of intracellular domains of NMDARs. In addition self-effects of full substitution of lithium for sodium were found. In some neurons lithium induced gradual elevation of intracellular calcium and large inward whole-cell currents, which could be the result of an inhibition of sodium-dependent glutamate uptake, glutamate accumulation in synaptic clefts and additional calcium entry via ionotropic glutamate receptor channels.

Disclosures: D.A. Sibarov: None. **E.E. Poguzhelskaya:** None. **P.A. Abushik:** None. **S.M. Antonov:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.24/E19

Topic: B.02. Ligand-Gated Ion Channels

Support: NSFC

Title: Modulation of NMDA receptors by TRPC6 and its function in synaptic plasticity

Authors: *H. SHEN[1], W. HU[2];
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Abstract: N-methyl-D-aspartate (NMDA) receptors play a key role in excitatory synaptic transmission, plasticity and neural development, and they also involved in neurodegenerative diseases and neuropsychiatric disorders. Therefore, regulation of the NMDA channel activity is critical for the physiological homeostasis and the pathological process of these diseases. Our work focuses on the modulation of NMDA receptors by pharmacological and endogenous

regulatory factor, for example, A. bidentata polypeptides (ABPP), an important constituent, which separated from the aqueous extract of A.bidentata Blume. Here, we reported that the canonical transient receptor potential channel 6 (TRPC6), a member of the TRPC family, can regulate the NMDA-induced current in primary cultured hippocampal neurons. Furthermore, TRPC6 can modulate the induction of the long term plasticity in CA3-CA1 synapses, and this function might involve in some mental diseases, such as depression and anxiety.

Disclosures: H. Shen: None. **W. Hu:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.25/E20

Topic: B.02. Ligand-Gated Ion Channels

Support: Ittner Foundation

Title: NMDA receptor antagonist-induced gamma neuronal oscillations are augmented in the GluN-2C knockout mouse; implications for schizophrenia.

Authors: *Z. MAO[1], Y. ZHANG[2], K. SAPKOTA[3], H. ALSAAD[3], S. DRAVID[4], D. MONAGHAN[3];
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Abstract: NMDA receptors appear to be involved in many neuropsychiatric diseases such as schizophrenia, autism spectrum disorder, bipolar disorder, and others. Their role in these disorders may be through causing a disruption in neuronal oscillations. Human studies have demonstrated increased basal gamma oscillatory activity in schizophrenics and reduced evoked oscillations. Similarly, NMDAR antagonists such as ketamine, and PCP augment basal neuronal oscillations in the gamma (and other frequency bands) while also mimicking the behavioral symptoms of schizophrenia. To identify the receptor subunits responsible for NMDAR antagonist modulation of neuronal oscillations, we examined the ability of the commonly used NMDAR antagonists (ketamine, PCP, and memantine) to stimulate neuronal oscillations in wildtype (WT) and GluN2C-knockout (GluN2C-KO) mice. Basal neuronal oscillations were measured by electrocorticography (ECoG) before and after drug administration in WT and GluN2C-KO mice. Frequency power spectrum analysis indicated that each of these NMDAR antagonists induced a larger increase in the gamma band's (30-100 Hz) oscillatory power in the cerebral cortex in GluN2C-KO mice compare to WT mice. Examination of 10 Hz bands

indicated that ketamine-enhanced power was significantly larger in the GluN2C-KO mice compared to WT mice in the 30-40, 40-50, and 50-60 Hz frequency bands. These results contrast with our recent findings that ketamine is not able to augment gamma oscillations in the GluN2D-KO mouse (Sapkota et al., JPET 356:702-711, 2016). Hence, GluN2C and GluN2D subunits appear to have significant and distinct contributions to the modulation of gamma oscillations by NMDA receptors.

Disclosures: Z. Mao: None. **Y. Zhang:** None. **K. Sapkota:** None. **H. Alsaad:** None. **S. Dravid:** None. **D. Monaghan:** None.

Poster

396. NMDA Receptors II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 396.26/E21

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01MH085666

Title: Psd95 deficiency alters nmda and ampa receptor expression and function during development in the prefrontal cortex

Authors: *A. COLEY[1], W.-J. GAO[2];
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Abstract: Postsynaptic density protein– 95 (PSD-95) is a major regulator in the maturation of excitatory synapses by interacting and trafficking N-methyl-D-aspartic acid receptors (NMDAR's) and α-amino-3-hydroxy-5-methyl-4-isox-azoleproprionic acid receptors (AMPAR's) to the postsynaptic membrane of the dendritic spine. PSD-95 and NMDA dysfunction have been associated with cognitive and learning deficits connected to psychiatric disorders such as autism and schizophrenia. Using a PSD-95 knockout mouse model (PSD-95$^{-/-}$), we examined how PSD-95 deficiency affects NMDAR and AMPAR expression and function in the dorsomedial prefrontal cortex (dmPFC) at postnatal days 21, 35 and 70, i.e., juvenile, adolescent and adult periods, respectively. We found significant increases in total protein levels of NMDAR subunits NR1, NR2B and NR3A, accompanied with a trending decrease in AMPAR subunit GluR1 during adolescence. However, no significant changes in protein levels were observed during juvenile and adult time points, indicating that the adolescence age range is a critical period at which PSD-95 influences NMDAR and AMPAR expression levels in the dmPFC. Whole-cell patch clamp recordings of NMDA- and AMPA-mediated currents were also

performed from the layer 5 pyramidal neurons of the dmPFC and NMDA/AMPA ratios were calculated. We found a significant increase in NMDA-mediated current and a decrease in AMPA-meditated current in PSD-95$^{-/-}$ mice during adolescence. In addition, PSD-95$^{-/-}$ mice show a decrease in NMDAR-mediated current decay compared to control mice, which is indicative of the significant increase in NR3A protein expression levels. Our data suggest that the increase in NMDAR expression and function and a decrease in AMPAR are representative of a a potential increase in "silent synapses" or disrupted synaptic formation. This study will be used to describe the importance of PSD-95 during dendritic spine development in the dmPFC and its potential association in the pathogenesis of cognitive and learning deficits in autism and/or schizophrenia.

Disclosures: A. Coley: None. **W. Gao:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 397.01/E22

Topic: B.02. Ligand-Gated Ion Channels

Support: Wellcome Trust

Title: Novel AMPA receptor modulators with an enhanced safety profile

Authors: *J. R. ATACK[1], P. BESWICK[1], M. HERD[2], N. UPTON[3], D. SPANSWICK[4], R. PORTER[5], M. GOSLING[1], J. LAMBERT[2], S. E. WARD[1];
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Abstract: Dysfunction of glutamatergic neurotransmission is thought to underlie a variety of psychiatric disorders such as schizophrenia and attention-deficit hyperactivity disorder. Since the α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA) subtype of ionotropic glutamate receptors mediate the majority of fast excitatory neurotransmission within the CNS, then modulation of AMPA receptors (AMPAR) is an attractive potential therapeutic approach. More specifically, compounds that are AMPAR positive allosteric modulators (PAMs) have been designed to enhance AMPAR function in disorders associated with glutamatergic hypofunction. Of these compounds, arguably the best known are the AMPAkine class of compounds, typified by CX516, CX691 and CX717. An issue with compounds that amplify the function of AMPARs is the potential for hyperexcitability, resulting in a proconvulsant or convulsant liability. We

have recently identified a novel class of compounds that are AMPAR PAMs that are devoid of any proconvulsant liability. Hence, these compounds potentiate AMPA currents in a human recombinant AMPAR (GluA2)-expressing cell line and rat primary neurons but are not proconvulsant in mouse hippocampal brain slices optimised to detect proconvulsant activity by reducing the extracellular Mg^{2+} concentration. Moreover, *in vivo* these compounds do not lower the seizure threshold, and are therefore not proconvulsant, in a rat maximal electroshock seizure threshold assay at doses of 300 mg/kg p.o. These compounds also enhance AMPA-mediated currents in a rat *in vivo* electrophysiology assay and enhance cognitive performance in the rat novel object recognition assay. Three of these compounds have been selected for further development and are currently undergoing preclinical safety and toxicity assays ready for first-in-human testing.

Disclosures: J.R. Atack: None. **P. Beswick:** None. **M. Herd:** None. **N. Upton:** None. **D. Spanswick:** None. **R. Porter:** None. **M. Gosling:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enterprise Therapeutics. **J. Lambert:** None. **S.E. Ward:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 397.02/E23

Topic: B.02. Ligand-Gated Ion Channels

Title: Pharmacological characterisation of the clinical AMPA receptor positive allosteric modulator [N-[(2S)-5-(6-fluoro-3-pyridinyl)-2,3-dihydro 1H-inden-2-yl]-2-propanesulfonamide]

Authors: *S. WARD[1], P. BESWICK[1], M. H. HARRIS[2], N. CALCINAGHI[3], J. GARTLON[2], F. GRAZIANI[3], L. LACROIX[2], S. MOK[2], B. OLIOSI[3], J. PARDOE[2], K. STARR[2];
[1]Univ. of Sussex, Brighton, United Kingdom; [2]GlaxoSmithKline, Harlow, United Kingdom; [3]GlaxoSmithKline, Verona, Italy

Abstract: Glutamate and its receptors have been of longstanding interest since dysfunction of the glutamatergic signalling pathway has been associated with the pathophysiology of several psychiatric and neurological disorders. The research on AMPA receptor positive allosteric modulators offers opportunities to modulate fast excitatory synaptic transmission and thus identify potential therapeutic agents for a range of disorders. The field of AMPAR modulators continues to be a dynamic area of drug discovery with a pronounced diversification of the chemotypes explored in recent years, however no molecules have yet progressed beyond preliminary Phase 2 evaluation.We report the full pharmacological characterisation and

translational characterisation of [*N*-[(2*S*)-5-(6-fluoro-3-pyridinyl)-2,3-dihydro 1*H*-inden-2-yl] -2-propanesulfonamide] a molecule which is a novel, potent and selective AMPA receptor positive allosteric modulator, currently in Phase 1 investigation. The supporting data to be presented demonstrate that the molecule is a positive allosteric modulator of the AMPA receptor and also that it was selective versus NMDA and kainate glutamate receptors. This potentiation was independent of subunit or orthologue composition in recombinant receptors, and was also demonstrated in rat native receptors. In vivo, the molecule was able to reverse a time delay-induced deficit in novel object recognition in rats after both acute and sub-chronic dosing. Sub-chronic dosing reduced the minimum effective dose from 0.3 mg/kg to 0.03 mg/kg. Efficacy was observed in additional cognition models. In side-effect profiling studies, there were no significant changes in the maximal electroshock threshold test at doses below 10 mg/kg. In conclusion, [*N*-[(2*S*)-5-(6-fluoro-3-pyridinyl)-2,3-dihydro 1*H*-inden-2-yl] -2-propanesulfonamide] is a potent and selective AMPAR modulator which exhibits cognition enhancing properties in several rat behavioural models superior to other molecules which have previously entered clinical evaluation and with a safety profile supportive of progression to clinical testing.

Disclosures: **S. Ward:** None. **P. Beswick:** None. **M.H. Harris:** None. **N. Calcinaghi:** None. **J. Gartlon:** None. **F. Graziani:** None. **L. Lacroix:** None. **S. Mok:** None. **B. Oliosi:** None. **J. Pardoe:** None. **K. Starr:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 397.03/E24

Topic: B.02. Ligand-Gated Ion Channels

Title: Identification and characterization of the binding pocket for negative allosteric modulators in AMPA receptors

Authors: *C. STENUM-BERG, C. L. THISTED, S. C. ABIEGA, A. S. KRISTENSEN; Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Kobenhavn O, Denmark

Abstract: AMPA receptors (AMPARs) are present throughout the CNS and mediate the majority of fast excitatory neurotransmission, and are involved in most brain functions. Abnormal AMPAR activity is implicated in neurological CNS diseases such as Alzheimer's, Parkinson's, epilepsy and psychiatric diseases. Recently, the first AMPAR-selective drug was approved in the form of perampanel for treatment of epilepsy. Perampanel belongs to the class of negative allosteric modulators (NAMs), inhibiting AMPAR activity via a non-competitive

mechanism. However, little is known about the location and structure of binding sites for perampanel or other prototypical AMPAR NAMs such as GYKI-53655 and CP-465,022. Identifying NAM binding sites and establishing NAM binding modes is prerequisite to understanding the molecular mechanism that underlies negative allosteric modulation of AMPAR function and is important for future development of other NAMs.
This project aims to characterize the binding sites and binding modes of perampanel and other NAMs. AMPARs are tetrameric assemblies of structurally closely related GluA1 to GluA4 subunits. The subunit architecture is highly modular with two large extracellular domains - the amino-terminal domain (ATD) and the ligand-binding domain (LBD) - that connect via three flexible linkers (denoted S1-M1, S2-M3, and S2-M4) to a transmembrane domain (TMD). Previous work has identified regions within the linker regions to harbor specific residues that can control NAM functional potency (Balannik et al., 2005, Neuron). To further identify specific amino acid residues in these regions that participate in NAM interaction, mutational scanning was performed of the S1-M1 and S2-M4 linker regions. 43 point mutants were functionally and pharmacologically characterized using an intracellular calcium imaging assay of AMPAR activity in HEK293 cells and an enzyme-based surface expression assay. For the 32 mutants with intact receptor function, concentration-response curves were constructed for four prototypical NAMs, including perampanel, to identify mutant effects on NAM inhibitory potency (IC50). Mutations at 12 positions were found to induce a >5-fold change in IC50 of one or more NAMs. Mapping of these amino acid positions in the crystal structure of the GluA2 AMPAR (Sobolevsky et al., 2009, Nature) identify two major areas to be involved in NAM function. The key mutants for which NAM inhibition was altered by at least five-fold in the calcium imaging assay are being further characterized by two-electrode voltage clamp electrophysiology.The mutational dataset will be used to guide molecular modeling studies of NAM binding modes to the receptor.

Disclosures: **C. Stenum-Berg:** None. **C.L. Thisted:** None. **S.C. Abiega:** None. **A.S. Kristensen:** None.

Poster

397. AMPA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: SFB 746

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AB393/1-2

AB393/2-2

Title: Defective FRRS1l impairs AMPA-receptor biogenesis and causes severe intellectual disability.

Authors: J. SCHWENK[1], A. BRECHET[1], S. BOUDKKAZI[1], R. BUCHERT[2], G. ZOLLES[1], K. SIQUIER-PERNET[5], W. BILDL[1], A. SAADI[6], C. BOLE-FEYSOT[5], P. NITSCHKE[5], N. AL-SANNA'A[7], A. REIS[3], A. KULIK[1], U. SCHULTE[1], L. COLLEAUX[5], R. ABOU JAMRA[4], *B. FAKLER[1];
[1]Inst. of Physiol., Freiburg, Germany; [2]Univ. of Erlangen, [3]Inst. of Human Genet., Erlangen, Germany; [4]Inst. of Human Genet., Leipzig, Germany; [5]INSERM UMR 1163, Inst. IMAGINE, Paris, France; [6]Dept. de Neurologie, Algers, Algeria; [7]Dharan Hlth. Ctr., Saudi Aramco, Saudi Arabia

Abstract: AMPA-type glutamate receptors (AMPARs), key players in excitatory neuro-transmission in the brain, are macromolecular complexes whose properties and cellular functions are determined by the co-assembled constituents of their proteome. Here we identify AMPAR assemblies containing FRRS1l/C9orf4 that are restricted to the endoplasmic reticulum and lack the core-subunits typical of AMPARs in the plasma membrane. Bi-allelic mutations in the human FRRS1l gene cause severe intellectual disability, cognitive impairment, speech delay and epileptic activity. Virus-directed deletion or overexpression of FRRS1l in adult rats alters the number of surface AMPARs in individual synapses resulting in decreased or increased amplitudes of the excitatory postsynaptic currents (EPSC) without effects on their time courses. Our results provide insight into the early biogenesis of AMPARs and demonstrate its impact on synaptic transmission and brain function.

Disclosures: **J. Schwenk:** None. **A. Brechet:** None. **S. Boudkkazi:** None. **R. Buchert:** None. **G. Zolles:** None. **K. Siquier-Pernet:** None. **W. Bildl:** None. **A. Saadi:** None. **C. Bole-Feysot:** None. **P. Nitschke:** None. **N. Al-Sanna'a:** None. **A. Reis:** None. **A. Kulik:** None. **U. Schulte:** None. **L. Colleaux:** None. **R. Abou Jamra:** None. **B. Fakler:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 397.05/E26

Topic: B.02. Ligand-Gated Ion Channels

Title: Identification of a novel protein that regulates endosome pH, AMPAR trafficking and synaptic function

Authors: *A. J. KALLARACKAL[1], J. MELLEM[2], D. M. MADSEN[2], V. MARICQ[2];
[1]Dept Biol., [2]Biol., Univ. of Utah, Salt Lake City, UT

Abstract: Dynamic regulation of glutamatergic AMPA receptors is crucial for synaptic function, behavior and cognition. We recently demonstrated that AMPARs are actively transported to synapses along microtubules via kinesin motors and that this transport is regulated by CaMKII. We now address how AMPAR exocytosis and endocytosis is regulated following delivery. In a forward genetic screen, we identified a novel mutant that increased the pH of AMPAR transport vesicles. Through whole genome sequencing we identified that the phenotype was due to a point mutation in a relatively uncharacterized gene that has been implicated in neurodegenerative disease. Furthermore, we found that this alkalized pH is mediated by a sodium-proton exchanger. In both mutants, we found defects in synaptic function that are a consequence of altered exocytosis. These findings provide new mechanistic insights into glutamatergic signaling and the consequences of altered regulation of intracellular pH.

Disclosures: A.J. Kallarackal: None. **J. Mellem:** None. **D.M. Madsen:** None. **V. Maricq:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

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Program#/Poster#: 397.06/E27

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH DC004450

NIH NS028901

NIH F32DC014878

Title: Mechanisms underlying slow AMPA-receptor mediated current at mossy fiber-unipolar brush cell synapse

Authors: H.-W. LU[1], *T. S. BALMER[2], G. E. ROMERO[3], L. O. TRUSSELL[2,1];
[1]Vollum Inst., [2]Oregon Hearing Res. Ctr., [3]Physiol. & Pharmacol., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Most AMPA-receptor (AMPAR) mediated EPSCs in the nervous system last only a few milliseconds. However, at mossy fiber-unipolar brush cell (UBC) synapses in the cerebellum, a slow AMPAR-mediated EPSC lasting for hundreds of milliseconds emerges after fast EPSCs end, leading to a prolonged depolarization. The slow current has been hypothesized to reflect the bell-shaped steady-state dose-response for desensitized AMPA receptors produced by slowly declining [glutamate] in the synaptic cleft. Here we provide evidence for this process using glutamate uncaging, synaptic stimulation, rapid glutamate application, and γ-2 TARP mutant mice (stargazer). Recordings were made from UBCs in WT or stargazer mouse brain slices containing cerebellar lobe X. A 2-ms uncaging of 1 mM MNI-glutamate at the synapse generated a fast-then-slow current similar to that evoked by synaptic stimulation. When the same uncaging pulse was delivered 15 ms after synaptic stimulation, the peak of the fast uncaging current was attenuated by 89 ± 1 % (n = 5), showing that AMPA receptors are greatly desensitized by synaptically released glutamate. Desensitization of the peak response recovered during the course of slow current phase with a time constant of ~296 ± 31 ms (n = 5), indicating that [glutamate] was slowly decreasing in the synaptic cleft. Similar desensitization was also observed by paired-uncaging pulses (recovery time constant: 465 ± 55 ms, n = 6). Application of 5% dextran to the bath reduced the decay of the slow EPSC (553 ± 140 to 830 ± 241 ms, n = 4), further supporting the role of slow glutamate diffusion in this process. Rapid-application of different concentrations of glutamate onto dissociated UBCs showed that the steady-state response peaks at ~32 μM and decreases as [glutamate] increases (response at 1 mM vs 32 μM: 70 ± 6 %, n = 11), suggesting that the cleft [glutamate] at the peak of the slow EPSC is around 32 μM. This bell-shaped dose response curve was absent in dissociated UBCs from stargazer mice (response at 1 mM vs 32 μM: 95 ± 3 %, n = 9), and the slow EPSC evoked by synaptic stimulation was significantly smaller compared with wild-types (5.2 ± 0.4 vs 13.5 ± 1.5 pA, n = 13 and 17, respectively), suggesting that γ-2 TARPs enhance the steady-state response at the mid-micromolar range. These effects could be reproduced using a kinetic model which stabilized open states of less-than-fully occupied AMPA receptors during slow transmitter transients. Taken together, we provide the evidence that the slow EPSC at mossy fiber-UBC synapse is due to slow diffusion of glutamate in the synaptic cleft, that it is mediated by desensitized AMPARs, and that TARPs regulate this process.

Disclosures: **H. Lu:** None. **T.S. Balmer:** None. **G.E. Romero:** None. **L.O. Trussell:** None.

Poster

397. AMPA Receptors

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Program#/Poster#: 397.07/E28

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG grant HO 1118/11-2 to M.H.

Title: Claudins: An unexpected source for more tetraspanning proteins acting as transmembrane AMPA receptor modulatory proteins

Authors: S. HAERING[1], S. BHATTACHARYA[2], M. ASLAM[3], T. STRASDEIT[4], J. VON ENGELHARDT[3], S. F. TRAYNELIS[2], *M. HOLLMANN[5,4];
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Abstract: The ionotropic glutamate receptors (iGluRs) are known to play a major role in excitatory signal transmission in the central nervous system. Although these receptors are able to function on their own, many interacting and modulating auxiliary subunits have been found. Some of them influence biophysical properties of iGluRs while others modulate the trafficking of the receptors to the plasma membrane. The most commonly known proteins are the transmembrane AMPA receptor regulatory proteins (TARPs), cornichon homologs (CNIH), neuropilin- and tolloid-like proteins (NETOs), cysteine-knot AMPAR modulating protein (CKAMP44), SynDigI, suppressor of lurcher-1 (SOL-1), and GSG1L.
We recently identified another class of proteins as possible AMPA receptor auxiliary subunits, the claudins. While generally known as tight junction proteins that seal passageways within and in between plasmamembranes, claudins show high structure and sequence homology with the TARP proteins. Of the 22-24 different claudin genes identified in rat, mouse, and humans, few have been functionally characterized. We show in two different expression systems, *Xenopus* oocytes and HEK-293 cells, that certain claudins, claudin-20 and claudin-24 in particular, potentiate the current amplitude and modulate the desensitization of certain AMPA receptors. The electrophysiological properties of other iGluR subtypes are not affected, and other claudins do not show this modulatory action. We identified unexpected amplitude potentiations at first in the oocyte system on GluA1 and GluA2 receptors, with effects being more pronounced with flip than with flop splice variants and with R than with Q editing variants. It still remains to be tested whether this exquisitely variant-specific effect, which is reminiscent of the TARP γ5, will be similarly variant-specific when tested in HEK cells.
We further show that both claudin-20 and claudin-24 are expressed in the brain, particularly in

the granule cell layers of the cerebellum and the olfactory bulb, and claudin-24 in addition to a lesser degree in the principal cells of the hippocampus. Finally, we used FRET after acceptor bleaching to demonstrate that claudin-20 interacts directly with GluA1.
Supported by DFG grant HO 1118/11-2 to M.H.

Disclosures: **S. Haering:** Other; Department of Biochemistry I - Receptor Biochemistry, Ruhr University Bochum, Bochum, 44780, Germany. **S. Bhattacharya:** None. **M. Aslam:** None. **T. Strasdeit:** Other; RUB Research SchoolPlus, Ruhr University Bochum, 44780 Bochum, Germany, Graduate School of Chemistry and Biochemistry, Ruhr University Bochum, 44780 Bochum, Germany. **J. Von Engelhardt:** None. **S.F. Traynelis:** None. **M. Hollmann:** None.

Poster

397. AMPA Receptors

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 397.08/E29

Topic: B.02. Ligand-Gated Ion Channels

Support: Health Research Council of New Zealand

University of Otago Anatomy Department

DAAD short term research grant for PhD candidates and young academics and scientists

Title: Secreted amyloid precursor protein-alpha regulates synthesis of the AMPA receptor subunit GluA1

Authors: ***M. K. ELDER**[1], K. PEPPERCORN[2], S. TOM DIECK[4], L. KOCHEN[4], E. SCHUMAN[4], W. TATE[2], C. ABRAHAM[3], J. WILLIAMS[1];
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Abstract: The amyloid precursor protein, best known as the precursor molecule to toxic beta amyloid, can also liberate a neuroprotective secreted fragment called secreted amyloid precursor protein-alpha (sAPPα). This fragment, derived from proteolysis by α-secretase enhances long-term potentiation (LTP) and spatial memory. The mechanism/s by which sAPPα facilitates these functions are as yet unknown. However, previously we have shown that sAPPα enhances synaptic protein synthesis, and as de novo protein synthesis is a requirement for persistence of both memory and LTP, we hypothesized that sAPPα may upregulate the synthesis of key proteins involved in these processes. As regulation of the ionotropic α-amino-3-hydroxy-5-

methyl-4-isoxazolepropionic acid (AMPA)-subtype of glutamate receptors is critical for LTP, in the current study we investigated the effect of sAPPα on the synthesis of the GluA1 and GluA2 subunits of this receptor. Following 2 h incubation of hippocampal neuronal primary cultures with sAPPα (1 nM), fluorescent non-canonical amino acid tagging with proximity ligation assays (PLA-FUNCAT) was used to label newly synthesized GluA1 or GluA2 subunits. Here, a methionine analogue bound to an azide group (azidohomoalanine, AHA) was co-incubated with sAPPα and incorporated into newly synthesized proteins in methionine-free media. Using click chemistry, a biotin-bound alkyne was linked to the AHA incorporated in new proteins, and this complex was recognized with high specificity using antibodies and PLA probes.
Using this technique, we revealed a cell-wide increase in GluA1 synthesis in response to sAPPα ($p < 0.0005$, Kruskal Wallis test), with two-fold more GluA1 visible in the dendritic compartment following treatment ($p < 0.05$). No effect was observed on the level of GluA2 synthesis in response to sAPPα. Together, these results reveal a possible mechanism of action, where sAPPα mediates an increase in GluA1 synthesis, enhancing availability of the subunit and therefore facilitating LTP and memory formation.

Disclosures: **M.K. Elder:** None. **K. Peppercorn:** None. **S. tom Dieck:** None. **L. Kochen:** None. **E. Schuman:** None. **W. Tate:** None. **C. Abraham:** None. **J. Williams:** None.

Poster

397. AMPA Receptors

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Program#/Poster#: 397.09/E30

Topic: B.02. Ligand-Gated Ion Channels

Support: Canadian Institutes of Health Research

NIH RO1

HHMI

Title: Extensive phosphorylation of AMPA receptors in neurons

Authors: ***N. K. HUSSAIN SHULER**, G. H. DIERING, S. HEO, B. LIU, R. L. HUGANIR; Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Changes in the synaptic targeting of AMPA receptors (AMPARs) is a fundamental mechanism controlling synaptic strength during long-term potentiation/depression and homeostatic scaling. AMPAR trafficking is controlled by protein-protein interactions as well as post-translational modifications. Phosphorylation of the GluA1 AMPAR subunit at S845 and

S831 play especially important roles during synaptic plasticity. Recent controversy has emerged regarding the extent to which GluA1 phosphorylation may contribute to synaptic plasticity. Here we used a variety of methods to measure the population of phosphorylated GluA1-containing AMPARs in cultured primary neurons and mouse forebrain. We demonstrate that phosphorylated GluA1 represents large fractions of the total population under basal and stimulated conditions in vitro and in vivo. Our results support the large body of research indicating a prominent role of GluA1 phosphorylation in synaptic plasticity.

Disclosures: **N.K. Hussain Shuler:** None. **G.H. Diering:** None. **S. Heo:** None. **B. Liu:** None. **R.L. Huganir:** None.

Poster

397. AMPA Receptors

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Support: BBSRC Grant BB/L502649/1

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MRC Grant MR/J012998/1

Title: The auxiliary subunit C9orf4 (FRRS1l) slows AMPAR recovery from desensitization

Authors: *S. PEARCE, M. FARRANT, S. G. CULL-CANDY;
NPP, Univ. Col. London, London, United Kingdom

Abstract: AMPA-type glutamate receptors (AMPARs) mediate the majority of fast excitatory neurotransmission in the mammalian CNS. These receptors are associated with various auxiliary proteins that affect their properties. Transmembrane AMPAR regulatory proteins (TARPs), the first identified AMPAR auxiliary subunits, have been shown to influence receptor trafficking, kinetics and pharmacology [1]. Additional auxiliary subunits have been identified, including the cornichon homologues CNIH-2 and CNIH-3, and the proteins CKAMP-44 and GSG1L, all of which individually influence AMPAR properties [2-5]. One interacting protein for which no functional effects have yet been described is the transmembrane protein ‘chromosome 9 open reading frame 4’ (C9orf4) (also known as ferric-chelate reductase 1-like protein; FRRS1l) [5]. Using patch-clamp recording and rapid glutamate application to excised patches, we have investigated the effects of C9orf4 co-expression on homomeric (GluA1) and heteromeric (GluA1/A2) AMPARs in tsA201 cells. C9orf4 slowed recovery from desensitization (induced by

100 ms application of 10 mM glutamate) without affecting other AMPAR properties. When the prototypical TARP stargazin (γ-2) was co-expressed, the effect of C9orf4 was lost and receptor properties resembled those of AMPARs associated with γ-2 alone. In cultured hippocampal neurons, overexpression of C9orf4 had no effect on mEPSC amplitude or frequency, suggesting either that it does not associate with native TARP-coupled receptors, does not alter their functional properties, or that C9orf4-associated AMPARs do not reach the synapse. Further experiments are required to determine the mechanism by which C9orf4 slows AMPAR recovery from desensitization, and its functional significance. 1. Jackson & Nicoll, *Neuron* 70, 178-199 (2011). 2. Schwenk *et al.*, *Science* 323, 1313-9 (2009). 3. von Engelhardt *et al.*, *Science* 327, 1518-22 (2010). 4. Shanks *et al.*, *Cell Reports* 1, 590-9 (2012). 5. Schwenk *et al. Neuron* 74, 621-33 (2012). Supported by the MRC (MR/J002976/1 to SGC-C and MF, MR/J012998/1 to MF and SGC-C) and BBSRC (BB/L502649/1 to SGC-C)

Disclosures: S. Pearce: None. **M. Farrant:** None. **S.G. Cull-Candy:** None.

Poster

397. AMPA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant RO1 HD061543

Title: Characterization of AMPAR auxiliary subunit GSG1L expression pattern and function using transgenic model systems

Authors: *A. KAMALOVA[1], E. ZAIKA[1], K. FUTAI[2], E. DELPIRE[1], T. NAKAGAWA[1]; [1]Mol. Physiol. and Biophysics, Vanderbilt Univ., Nashville, TN; [2]Dept. of Psychiatry, Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: AMPA receptors (AMPAR) are the predominant excitatory neurotransmitter receptors that mediate fast excitatory synaptic transmission in the brain. The density of AMPARs at the synapse is postulated to underlie a subset of synaptic plasticity. AMPAR auxiliary subunits are transmembrane regulatory proteins that selectively associate with AMPARs affecting various aspects of AMPAR life cycle from early trafficking to gating kinetics. GSG1L is a newly identified auxiliary subunit identified in our laboratory with unique AMPAR regulatory properties. GSG1L slows the rate of desensitization, similar to what was observed for the canonical transmembrane AMPAR regulatory proteins (TARPs). However, while TARPs speed the recovery from desensitization, GSG1L slows this process in heterologous systems.

Furthermore, a recent study demonstrated that GSG1L reduces the average single-channel conductance and enhances polyamine block of calcium permeable AMPARs. It is unknown, however, which brain regions and subset of neurons express GSG1L. To address this, we conducted histological analysis on a transgenic rat that expresses a lacZ reporter under endogenous GSG1L promoter. In this transgenic rat, a lacZ reporter disrupts the GSG1L gene by interrupting its normal RNA splicing event, producing a GSG1L KO. Using lacZ localization, we deduced the spatio-temporal expression pattern of GSG1L during postnatal development, which was revealed to be highly dynamic. While GSG1L expression was undetectable in hippocampus during early postnatal development, it increased in DG and CA3 regions in older animals. Expression was also high in L2/3 cortical neurons. The LacZ expression pattern in heterozygotes, which express half dose of GSG1L, was comparable to that of the homozygotes, which express negligible amount of GSG1L. The expression pattern deduced from lacZ staining is consistent with in situ hybridization data from Allen Brain Atlas. However, the current results extend our knowledge of GSG1L distribution by providing more detailed spatiotemporal analysis. In order to characterize GSG1L function in synapses, we then generated GSG1L KO mouse model owing to availability of homogenous genetic backgrounds and genetic tools. The phenotype is currently being characterized and progress will be presented at the meeting.

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Poster

397. AMPA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R21 MH102546

NIH Grant T32 GM008320

Title: Using high throughput screening methods to identify small molecule modulators that specifically target the GluA2-auxiliary subunit complex

Authors: *C. AZUMAYA[1], E. DAYS[2], P. VINSON[2], S. STAUFFER[3], G. SULIKOWSKI[4], D. WEAVER[3], T. NAKAGAWA[5];
[2]Vanderbilt Inst. of Chem. Biol., [3]Pharmacol., [4]Chem., [5]Mol. Physiol. and Biophysics, [1]Vanderbilt Univ., Nashville, TN

Abstract: AMPA receptors (AMPAR) are ligand gated ion channels that are critical to maintain normal synaptic plasticity. Because of their integral role in fast excitatory neurotransmission, AMPA receptor dysfunction is implicated in a variety of cognitive diseases ranging from developmental to degenerative. Attempting to potentiate or depress AMPAR activity is an inherently difficult balancing act between effective treatment and debilitating side effects. A newly explored avenue for increasing our ability to target subsets of AMPAR in the central nervous system is to identify compounds that target specific AMPAR-auxiliary subunit complexes. Here we report a high throughput screening-based pipeline that is able to identify compounds targeted to certain GluA2-auxiliary subunit combinations, specifically, GluA2-CNIH3 and GluA2-stargazin. These compounds will build upon the growing library of AMPAR-auxiliary subunit specific modulators which have so far all been targeted to TARP-8. Our screening protocol begins with a cell-based assay that utilizes a voltage sensing dye to identify changes in membrane potential of HEK cells co-expressing GluA2 and an auxiliary subunit. This primary screen was followed by a set of counterscreens to remove compounds working through undesired mechanisms. Our workflow was able to narrow an initial library of 40,000 compounds down to 54 hits that are specific for GluA2-stargazin or GluA2-CNIH3 expressing cell lines for further characterization using electrophysiology, fluorescence-based calcium flux assay, and co-immunoprecipitation assay. Candidate compounds from the initial screen have been identified as negative (NAM) and positive (PAM) allosteric modulators specific to either stargazin or CNIH3. Candidate compounds that affect both auxiliary subunit containing complexes, but not GluA2 alone, were also identified. Progress on further verification of these candidate compounds is underway. Our pipeline is a feasible way to identify compounds that are specific to many different iGluR-auxiliary subunit combinations, which could prove important to specific targeting of auxiliary subunit containing complexes in the lab and clinic. Differential expression patterns for auxiliary subunits throughout the central nervous system presents a rational approach to specifically target AMPARs in certain regions of the brain to decrease the potential of on-target side effects.

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Poster

398. Ca2+ Channels and Ca2+ Signaling

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Program#/Poster#: 398.01/E34

Topic: B.04. Ion Channels

Support: 2R01MH075916-05A

Title: Decreased palmitoylation of PSD-95 contributes to src mediated NMDA receptor hypoactivity in schizophrenia

Authors: *A. BANERJEE[1], A. SENGAR[2], J. KIM[3], R. RAY[4], K. BORGMANN-WINTER[5], M. SALTER[6], C.-G. HAHN[3];
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Abstract: Decreased GluN2 tyrosine phosphorylation and Src kinase hypoactivity are molecular signatures of disrupted NMDA receptor (NR) signaling in schizophrenia. Src hypoactivity can be traced to various molecular alterations in the DLPFC of schizophrenia subjects, one of which is through PSD-95. Here we report that increased PSD-95 within NR complex in schizophrenia results from its altered palmitoylation and trafficking into the PSD which in turn may reduce src activity in NR complex.
DLPFC tissues obtained from 15 pairs of SCZ and healthy subjects (UPenn SCZ brain bank) were subjected to subcellular fractionation, western blotting, immunoprecipitation, acyl biotin exchange assay for detecting palmitoylation, src activity assay with the PSD-95 inhibitory peptide (SAPIP), qPCR, RNA seq. SAPIP increased src activity ($p \leq 0.020$) in synaptic membrane (P2) by reducing interaction between src and PSD-95. PSD-95 protein was increased in ER/Golgi enriched fractions (P3) in schizophrenia ($p \leq 0.001$). C2'-GluN1: PSD-95 association, which increases membrane trafficking of PSD-95, was increased ($p \leq 0.004$) in the PSD in SCZ. Palmitoylation of PSD-95, that increases membrane transport of PSD-95 was decreased in both P3 ($p \leq 0.027$, $t=2.489$ $df=13$) and P2 ($p \leq 0.005$). RNA seq analysis on PSD-95 specific palmitoyltransferase ZDHHC 2, 7, 8 and 15 showed no significant changes in expression in SCZ whereas ZDHHC 3 transcript was decreased in SCZ ($p \leq 0.0006$) by ~10%. Our findings lead us to propose a model in which in SCZ decreased membrane trafficking of PSD-95 due to decreased palmitoylation leads to an alternate path of membrane transport via C2'-GluN1 and inhibition of src activity specifically within NR complexes.

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Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 398.02/E35

Topic: B.04. Ion Channels

Title: State-dependence alone does not provide a sufficient CNS margin with an orally efficacious Cav2 selective small molecule

Authors: *K. S. RATLIFF[1], K. KNOPP[2], J. SCHKERYANTZ[2], B. T. PRIEST[2], M. CLARK[2], R. CERNE[2], M. WAKULCHIK[2], B. HEINZ[2], M. WALKER[2], A. VANDERGRIFF[2], X. HUANG[2], M. J. VALLI[2], W. J. PORTER[2], J. K. REEL[2], D. LUFFER-ATLAS[2], T. JONES[2], R. M. A. SIMMONS[2], B. FORSTER[2], W. GUO[2], B. ADAMS[2], L. YANG[2], J. S. MCDERMOTT[2]; [1]Neurosci. Discovery, Indianapolis, IN; [2]Eli Lilly & Co., Indianapolis, IN

Abstract: The N-type voltage gated calcium channel, Cav2.2, has long been a target of interest for chronic pain drug discovery. This has been based principally on the clinical efficacy observed with ziconotide (ω-conotoxin-MVIIA), a potent and selective Cav2.2 inhibitor derived from the venom of a cone snail. Unfortunately, the clinical utility of ziconotide has been limited by both its intrathecal route of administration as well as a very narrow therapeutic margin. Typical side effects include CNS and baroreceptor deficits that manifest at, or just above therapeutic drug concentrations. This narrow therapeutic margin may be the result of the state-independent mechanism of action of ziconotide. The addition of state-dependence in a Cav2.2 directed therapy would selectively target more depolarized and/or rapidly firing nociceptors, and spare the more hyperpolarized Cav2.2 expressing neurons, perhaps diminishing some of the adverse effects. However, despite over a decade of small molecule drug discovery efforts, clinical proof of concept for this hypothesis has been elusive. In addition, ligands disclosed to date possess the desired state-dependence, but not the selectivity of ziconotide, as they are typically selective over other Cav family members, but equipotent for Cav2.1, 2.2, and 2.3. There is a single report showing a 20-fold preclinical CNS margin with a potent and state-dependent Cav2 selective small molecule, and while this is an improvement over ziconotide, this compound has apparently not progressed into clinical testing. Here we describe a potent and state-dependent Cav2 selective small molecule, 3302917, that is efficacious in multiple preclinical in vivo pain assays. The compound also achieved exposures in a rodent toxicity study sufficient for a 10-fold CNS margin determination, with ataxias being the primary observation. 3302917 also showed efficacy in a formalin assay in Cav2.2$^{-/-}$mice, and while this result would typically be interpreted as evidence of off-target effects, we believe that both the ataxias observed in the toxicity study and the efficacy in the Cav2.2 null mice provide evidence of Cav2.1 inhibition. Cav2.1 is highly expressed in the cerebellum and loss of function mutations in humans are linked to ataxias. In addition, Cav2.1 selective toxins have shown efficacy in the formalin model. While efficacious in multiple pain assays, 3302917 did not have the desired CNS margins to allow further development. We propose that future drug discovery efforts targeting Cav2.2 should incorporate not only a state-dependent mechanism of action, but also some degree of Cav2.1 selectivity.

Disclosures: **K.S. Ratliff:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **K. Knopp:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **J. Schkeryantz:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **B.T. Priest:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **M. Clark:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **R. Cerne:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **M. Wakulchik:** A.

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Poster

398. Ca2+ Channels and Ca2+ Signaling

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Topic: B.04. Ion Channels

Support: CIHR Grant Canada

Eyes high postdoctoral fellowship

AIHS postdoctoral fellowship

Title: L-type voltage gated calcium channels functionally couple with IKCa channels in CA1 pyramidal cells to generate the slow afterhyperpolarization

Authors: *G. SAHU, J. MICLAT, H. ASMARA, G. W. ZAMPONI, R. W. TURNER; Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Calcium activated potassium (KCa) channels control the frequency and pattern of spike discharge in hippocampal pyramidal neurons. The intermediate conductance KCa (IKCa) channel (KCa3.1) was recently reported to contribute to generation of the slow afterhyperpolarization (sAHP) (King et al. 2015). Voltage-gated calcium channels of the L-type (CaV1 family) have been reported to act as a primary calcium source for the sAHP, but these tests relied on dihydropyridine blockers that also block IKCa. The current study examined the ability for CaV1.2 or CaV1.3 channels to activate IKCa to contribute to the sAHP. Coexpression of CaV1 and KCa3.1 cDNA in tsA-201 cells revealed that either CaV1.2 or

CaV1.3 is sufficient to activate IKCa channels, with a close relationship between voltage-dependent activation of CaV1 currents and the magnitude of IKCa. Moreover, step commands that maximally activated CaV1 channel isoforms for 5-150 msec produced a graded activation of IKCa that lasted 1-5 sec, effectively recreating an IsAHP. Recordings from CA1 pyramidal cells in vitro in the presence of blockers against all CaV channel isoforms (except L-type), SK, BK, and Kv7 channels confirmed the activation of IsAHP that was sensitive to 1 μM TRAM-34, a selective IKCa blocker. Similarly, application of 500 nM isradipine determined in tsA-201 cells to block Cav1 but not KCa3.1 channels reduced IsAHP area and spike accommodation in CA1 pyramidal cells. Moreover, CaV1.3 but not CaV1.2 channels exhibited calcium-dependent facilitation when coexpressed with the scaffolding protein densin and CaMKII and activated with the 50 Hz protocol relevant to generating IsAHP. Further, siRNAs against densin reduced IsAHP area and calcium-dependent facilitation in cultured hippocampal pyramidal neurons. The CaV1-mediated activation of IKCa channels in pyramidal cells was blocked by internal 5 mM EGTA, suggesting a microdomain-based interaction.
The results suggest that L-type calcium channel isoforms, and more specifically CaV1.3, are sufficient to activate IKCa channels in a manner consistent with the sAHP in CA1 pyramidal neurons.
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Poster

398. Ca2+ Channels and Ca2+ Signaling

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Faculty PhD Stipend

Title: The $5HT_{2C}$ receptor decreases seizure susceptibility in the dorsal subiculum by inhibiting Ca_V3 ion channels

Authors: *A. PETERSEN[1], C. S. JENSEN[1], V. CRÉPEL[2], M. FALKERSLEV[1], J.-F. PERRIER[1];
[1]Copenhagen Univ., Copenhagen N, Denmark; [2]INSERM U901, INMED, Marseille, France

Abstract: The subiculum is the major output region of the hippocampal network. It receives inputs from CA1 and entorhinal cortex and sends projections to several cortical and subcortical areas. Under pathological conditions, epileptic seizures start from the subiculum before spreading to the temporal lobe. Since serotonin (5-HT) was reported to have an antiepileptic effect, we investigated if and how it modulates the activity of principal cells from the subiculum. In two *in vitro* rodent models for epilepsy, we found that 5-HT inhibits subicular seizures. To understand the underlying mechanism, we recorded the electrical activity of subicular pyramidal neurons with the whole-cell patch clamp technique in a slice preparation from the hippocampus of mice. In agreement with previous observations, we found that subicular neurons fired action potentials in bursts triggered by a low threshold calcium spike. Focal release of 5-HT induced by pressure or microiontophoresis inhibited the burst firing. The calcium current responsible for burst firing was recorded in voltage-clamp mode after blocking Na^+ and K^+ voltage gated ion channels. A puff application of 5-HT decreased the amplitude of a low-threshold transient inward current sensitive to mibefradil. These results suggest that 5-HT inhibits a current mediated by T-type (Ca_V3) Ca^{2+} channels. To corroborate our findings, we monitored the variations in Ca^{2+} concentration by loading recorded cells with the Ca^{2+} indicator FURA-2. We observed that burst firing evoked by depolarizing current pulses induced an increase in Ca^{2+} concentration. When 5-HT was puff-applied, the Ca^{2+} signal was attenuated in all compartments of the neuron (AIS, soma, dendrites). Puffing a $5\text{-}HT_{2C}$ agonist (WAY 629 or WAY161503) had the same inhibitory effect on the burst firing and Ca^{2+} current. Using immunohistochemistry, we found that $5\text{-}HT_{2C}$ receptors are expressed in the dendrites of subicular pyramidal neurons in the vicinity of $Ca_V3.1$ and $Ca_V3.3$. When tested on in vitro model for epilepsy, $5\text{-}HT_{2C}$ agonists had an inhibitory effect comparable to 5-HT. Finally, we tested if the inhibitory effect could be obtained by synaptic release. Using a mouse expressing channelrhodopsin under the promoter controlling tryptophan hydroxylase 2, we evoked synaptic release by applying blue light in in the subiculum. It consistently inhibited the bursting of principal cells.
Our data suggest that the anti-epileptic effect of 5-HT in subiculum is mediated by 5-HT2c receptors, which in turn inhibit the T-type calcium channels responsible for the burst firing of principal cells.

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Poster

398. Ca2+ Channels and Ca2+ Signaling

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Center for Stroke Research Berlin 01 EO 0801

Title: Crosstalk between $InsP_3R$ and TRPV4 in Ca^{2+} microdomains contributes to paclitaxel-induced neurotoxicity

Authors: *W. BOEHMERLE, P. HUEHNCHEN, C. HARMS, M. ENDRES;
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Abstract: Neurotoxic phenomena are among the most common side effects of cytostatic chemotherapy and affect a large number of patients. Paclitaxel is a common antineoplastic drug which frequently causes length dependent sensory polyneuropathy. Increasing evidence suggests, that altered intracellular calcium (Ca2+) signals play an important role in the pathogenesis of paclitaxel-induced peripheral neuropathy. In the present study, we examined in cultivated dorsal root ganglion cells the interplay between Ca2+ release channels in the endoplasmic reticulum (ER) and Ca2+ permeable channels in the plasma membrane in the context of paclitaxel mediated neurotoxicity. Confocal microscopy revealed that the inositol-trisphosphate receptor (InsP3R) type 1 was typically concentrated close to the plasma membrane which is in contrast to homogenous ER distribution. G protein-coupled designer receptors as well as Ca2+ imaging with Ca2+ sensitive dyes were then used to further elucidate phosphoinositide mediated Ca2+ signaling. This approach confirmed that InsP3 mediated Ca2+ signals originate close to the plasma membrane and are amplified by Ca2+ entry through TRPV4 channels. In addition, we could show that InsP3R1 and TRPV4 physically interact and are partially colocalized. In the context of paclitaxel-induced neurotoxicity, inhibition of the lipid kinase PIP5K1C upstream of InsP3 generation, NCS-1 mediated InsP3R modulation or blocking Ca2+ influx through TRPV4 channels prevented cell death. In summary, these results provide evidence for pathophysiologicaly relevant Ca2+-signaling microdomains linking the cation channel TRPV4 in the plasma membrane and InsP3Rs in the ER. Our findings underline the usefulness of designer receptors exclusively activated by designer drugs and suggest several potential strategies for prevention of paclitaxel-induced neuropathy.

Disclosures: **W. Boehmerle:** None. **P. Huehnchen:** None. **C. Harms:** None. **M. Endres:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 398.06/F1

Topic: B.04. Ion Channels

Support: NIH Grant AG041360

Title: Acute estrogen causes a prolonged alteration of intracellular calcium signaling in basal forebrain neurons of F344 rats

Authors: ***D. A. MURCHISON**, A. S. FINCHER, W. H. GRIFFITH;
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Abstract: Estrogenic signaling modulates neuronal function in numerous ways in a variety of brain regions. However, promising early results related to cognition and neuroprotection in animal models have not translated well into therapeutic approaches to health issues of post-menopausal women. Our lab has been exploring the neurophysiology of acute and chronic estrogen signaling in a rat model of reproductive aging in order to identify mechanisms that could be targeted to relieve age-related impairments. We have focused on basal forebrain (BF) cholinergic neurons because of their involvement in age-related cognitive decline. Previously, we found that age-related cognitive impairment in F344 male rats was associated with enhanced intracellular Ca^{2+} buffering and reduced inhibitory synaptic transmission in cholinergic BF neurons. In ovariectomized (OVX) reproductively senescent (RS) females, chronic estrogen treatment (OVX+E, 3 weeks subcutaneous pellet) restored Ca^{2+} buffering and inhibitory transmission to youthful levels. Furthermore, we observed that acute estrogen applications (100-200 nM 17-β estradiol) reduced inhibitory synaptic transmission, increased excitatory transmission, inhibited voltage-gated currents and activated intracellular Ca^{2+} signaling. Many of these acute effects persisted in aged females, but were lost by middle-age in males. Here, we report that disruptions of intracellular Ca^{2+} homeostasis arising from acute estrogen applications are reduced in young adult (4-6 mo) female rats, relative to young males or RS females. We used fura-2 fluorescent Ca^{2+} imaging on acutely dissociated BF neurons from young adult male and female rats, as well as, RS OVX and OVX+E females (14-17 mo) to examine the impact of acute estrogen on Ca^{2+} signaling. Up to 12 min following a 3 min exposure to acute estrogen, the amplitude of evoked Ca^{2+} transients was increased relative to controls in all test groups. The increases were: young female 25%, young male 58%, OVX 22% and OVX+E 37%. Similar

increases were seen in the baseline Ca^{2+} concentration 5-15 min following acute estrogen: young female 21%, young male 74%, OVX 104% and OVX+E 73%. Note that for these parameters, chronic estrogen did not restore the values to youthful levels. Preliminary experiments suggest that some of these effects may be mediated through L-type (Ca_V1) Ca^{2+} channels, as the antagonist nifedipine (10 μM) reduced the baseline concentration increase in young males from 57 nM to 28 nM. These findings support the idea that disruption of normal Ca^{2+} signaling in the brain by acute estrogen may be detrimental to cognitive function during aging.

Disclosures: D.A. Murchison: None. **A.S. Fincher:** None. **W.H. Griffith:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

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Program#/Poster#: 398.07/F2

Topic: B.04. Ion Channels

Support: Intramural grant support from Rajiv Gandhi Centre for Biotechnology, India

Post doctoral fellowship from Department of Biotechnology, Government of India

Doctoral fellowship from Kerala State Council for Science, Technology and Environment, Governemnet of Kerala, India

Title: Regulation of NMDA receptor phosphorylation by multiple calcium signaling pathways

Authors: *R. V. OMKUMAR, M. JOHN, M. KUMAR, J. JAMES, M. MAYADEVI;
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Abstract: Calcium influx through N-methyl-D-aspartate receptors (NMDAR) and voltage gated calcium channels (VGCC) plays major roles in postsynaptic signaling mechanisms. Eventhough these pathways converge at the level of free calcium release in the cytosol, they maintain specificity towards their respective targets that are further downstream. Calcium influx through different channels may differ in their levels and durations, in the physiological context during which they occur as well as in the subcellular regions where they occur. NMDAR subunit GluN2B is phosphorylated at Ser^{1303} (*J Biol Chem. 1996, 271, 31670-8*). Phosphoryation at this site is a prominent event in cell culture systems as well as *in vivo* (*Int. J. Neuropsychopharmacol., 2010, 13, 1255-1260*). Binding of CaMKII to GluN2B is inhibited by phosphorylation at this site (*J Biol Chem. 2000, 275, 23798-806*). The conductance of NMDAR channel also depends on the phosphorylation status of the site (*Cell 2010, 140, 222-234*). GluN2B-Ser^{1303} phosphorylation level is likely to be sensitive to calcium signaling since the

calcium sensitive kinases, CaMKII (*J Biol Chem. 1996, 271, 31670-8*) and PKC (*Mol. Pharmacol., 2001, 59, 960*) are known to phosphorylate this site. Protein phosphatase 1 dephosphorylates this site (*Neurochem. Int. 2012, 61, 961-5; PLoS One, 2012, 7, e34047*). Despite the available data, the functional significance of phosphorylation at this site is not completely understood. In this study, we explored whether calcium signaling through NMDAR and VGCC differed in their effect on phosphorylation status of GluN2B-Ser^{1303} in the rat *in vivo* model. VGCC was activated by intraperitoneal (IP) injection of the VGCC activator, BayK8644 and NMDAR was activated by intracerebroventricular (ICV) injection of NMDA in separate experimental groups. Subsequently, the levels of phospho-GluN2B-Ser^{1303} in the cortex and in the hippocampus were monitored by western blotting. We find that phosphorylation at this site increases in response to Ca^{2+}-influx through either channel. However the level of GluN2B remains largely unchanged indicating that the effect is brought about by kinases or phosphatases. The effects could be prevented by prior ICV administration of the specific blockers of these channels such as MK-801 for NMDAR and nifidipine for VGCC. The effect was also blocked by pretreatment of the animals with ICV administration of KN-93 indicating that it is mediated through CaM kinase. The levels of phosphorylation of GluR1 are also being analysed. We conclude that under *in vivo* conditions, calcium influx through either NMDAR or VGCC activates CaM kinase. This in turn phosphorylates GluN2B-Ser^{1303}.

Disclosures: R.V. Omkumar: None. **M. John:** None. **M. Kumar:** None. **J. James:** None. **M. Mayadevi:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 398.08/F3

Topic: B.04. Ion Channels

Support: NCTR/FDA

Title: Relationship between ketamine-induced toxicity and nmda receptor-mediated calcium influx in developing neurons

Authors: *C. WANG, F. LIU, T. A. PATTERSON, M. G. PAULE, W. SLIKKER, Jr;
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Abstract: Ketamine, a noncompetitive NMDA receptor antagonist, is used as a general anesthetic and recent data suggest that anesthetics can cause neuronal damage when exposure occurs during development. To elucidate the underlying mechanisms associated with ketamine

neurotoxicity, neural cells were harvested from the forebrain of newborn rats and neural stem cells were isolated from gestational day 16 rats. To determine the effect of ketamine on developing neurons and undifferentiated neural stem cells, cultures were exposed to 10 μM ketamine for 24 hours. Ketamine exposure resulted in elevated NMDA receptor (NR1) expression in primary cultures, and enhanced damage of developing neurons including those differentiated from the neural stem cells. However, the viability and proliferation rate of neural stem cells were not significantly affected after ketamine exposure.
Since NMDA receptor-regulated ion channels are known to be highly permeable to calcium, a study to monitor the presence of, and/or changes in, intracellular calcium concentrations would be informative. Calcium imaging data indicated that 50 μM NMDA did not cause a significant influx of calcium in typical neural stem cells; however, it did produce an immediate elevation of intracellular free Ca^{2+} $[Ca^{2+}]_i$ in neurons differentiated from the same neural stem cells. NMDA (50 μM) also produced an elevation in $[Ca^{2+}]_i$ in both control and ketamine-exposed neurons in the primary cell cultures, and a significant increase in $[Ca^{2+}]_i$ was detected in ketamine-exposed neurons compared with control neurons after NMDA stimulation.
These findings suggest that prolonged exposure of developing neurons to ketamine produces an increase in NMDA receptor expression (compensatory up-regulation) which allows for a higher/toxic influx of calcium into neurons once ketamine is removed from the system, leading to neuronal cell death likely due to elevated reactive oxygen species generation. The absence of functional NMDA receptors in cultured neural stem cells may help to explain why clinically-relevant concentrations of ketamine did not affect undifferentiated neural stem cell viability.

Disclosures: C. Wang: None. **F. Liu:** None. **T.A. Patterson:** None. **M.G. Paule:** None. **W. Slikker:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 398.09/F4

Topic: B.04. Ion Channels

Title: Suppression of peripheral sympathetic activity underlies agmatine-mediated hypotension

Authors: *Y. KIM[1,2], S. CHUNG[2];
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Abstract: Agmatine is highly expressed in endothelial cells and vascular smooth muscle cells. It plays a crucial role in regulating blood pressure via the modulation of peripheral vascular tone.

Although several mechanisms have been suggested to explain agmatine-induced hypotension, the precise mechanism remains to be elucidated. We investigated the effect of agmatine activation on smooth muscle contraction evoked by electrical field stimulation (EFS) in the superior mesenteric artery. In the present study, agmatine suppressed neurogenic contractions evoked by EFS in endothelium-denuded superior superior mesenteric arterial strips but did not affect contraction elicited by the external application of noradrenaline (NA). In addition, ω-conotoxin GVIA (CgTx), a selective N-type Ca^{2+} channel blocker, significantly inhibited EFS-evoked contraction, and this blockade almost completely occluded the suppression of EFS-evoked contraction by PAR-2 agonists. These findings demonstrate that activation of PAR-2 suppresses peripheral sympathetic outflow by modulating N-type Ca^{2+} channel activity, which are located in peripheral sympathetic nerve terminals, and are involved in PAR-2-induced hypotension.

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Key words: agmatine; Hypotension; Peripheral sympathetic output; N-type Ca^{2+} channel; Mesenteric artery

Disclosures: Y. Kim: None. **S. Chung:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

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Program#/Poster#: 398.10/F5

Topic: B.04. Ion Channels

Support: CIHR MOP 86599

CIHR MOP 133602

CRC to EFS

Title: Characterization of the P-type voltage-gated calcium channel in chick

Authors: *Q. LI, B. ELLIOTT, E. F. STANLEY;
Krembil Res. Inst., Toronto, ON, Canada

Abstract: Transmitter release is gated primarily by P-type voltage-gated calcium channels at most mammalian synapses, while N-type channels play a lesser role. Previous research has

shown that at chick synapses (Staney and Goping, 1991) and other lower vertebrates (Thaler et al., 2001), transmission is gated almost exclusively by N-type voltage-calcium channels (CaVs). There is little published evidence to support the existence of P-type CaVs in avians, only fragmented sequences for a few species have been predicted on the NCBI database, including a small (171 amino acid) sequence for chicken (UniProtKB/Swiss-Prot: O73705.1). These findings led us to search for and characterize the P-type CaV in the chicken nervous system. We have used RT-PCR applied to whole chick brain cDNA to generate a full length P-type sequence. This sequence enabled us to design a chick-specific antibody targeted against the C-terminal of the channel. We used this to investigate the distribution of the channels in the chick brain using immunocytochemistry. We have also identified putative binding regions for ω-conotoxin GVIA and ω-agatoxin IVA. Interestingly, while the channel includes the transmembrane domains, the C-terminal is truncated and lacks a typical DxWC terminus. While we cannot as yet conclude any functional details about the channel, we do show that it is expressed in the chicken nervous system.

Disclosures: Q. Li: None. **B. Elliott:** None. **E.F. Stanley:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

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Program#/Poster#: 398.11/F6

Topic: B.04. Ion Channels

Support: NIH/NINDS grant NS087068

Title: Examination of the effects of NCLX knock-out on mitochondrial and cytosolic Ca^{2+} signaling in hippocampal neurons

Authors: Z. LIN, J. RYSTED, A. GNANASEKARAN, *Y. M. USACHEV;
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Abstract: Mitochondrial Ca^{2+} transport plays an important role in regulating various physiological and pathological processes in neurons including excitability, synaptic transmission, ATP synthesis, gene regulation and neuronal response to excitotoxic stress. Recently, NCLX ($Na^+/Li^+/Ca^{2+}$ exchanger, also known as SLC8b1) has been identified as an essential molecular component of mitochondrial Na^+/Ca^{2+} exchange, the system that mediates Ca^{2+} extrusion from the mitochondrial matrix. However the role of NCLX in neurons is not well understood. Here, we used NCLX knock-out (KO) mice (Jackson Lab, C57BL6 background) to examine the role of NCLX in the control of mitochondrial and cytosolic Ca^{2+} signaling in hippocampal neurons. We

simultaneously monitored cytosolic and mitochondrial Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mt}$, respectively) using Fura-2 and mitochondria-targeted Ca^{2+} indicator mtLAR-GECO1.2 as previously described (Wu et al., Biochem J, 2014). We found that NCLX KO increased the amplitude of $[Ca^{2+}]_{mt}$ elevation (by ~2-fold) produced by strong depolarization (50 mM KCl, 30 s) and also slowed its recovery toward the baseline by ~two-fold compared to hippocampal neurons from WT mice. In contrast, NCLX KO did not have a significant effect on either amplitude or duration of $[Ca^{2+}]_{mt}$ responses evoked by mild depolarization (20 and 30 mM KCl, 30 s) or by 100 μM glutamate (30 s). NCLX KO also did not significantly affect depolarization- or glutamate-induced $[Ca^{2+}]_{cyt}$ changes. A characteristic feature of NCLX is that it can use Li^+ instead of Na^+ to transport Ca^{2+}. Notably, we found that replacement of Na^+ with Li^+ halted Ca^{2+} removal from mitochondria in WT hippocampal neurons. In contrast, Na^+ replacement with Li^+ did not slow Ca^{2+} extrusion from mitochondria in WT sensory dorsal root ganglion (DRG) neurons. These results suggest that NCLX mildly contributes to mitochondrial Ca^{2+} transport in hippocampal neurons under certain experimental conditions (e.g., strong depolarization), and that its role in mitochondrial Ca^{2+} transport may differ between central and peripheral neurons.

Disclosures: Z. Lin: None. **J. Rysted:** None. **A. Gnanasekaran:** None. **Y.M. Usachev:** None.

Poster

398. Ca2+ Channels and Ca2+ Signaling

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Program#/Poster#: 398.12/F7

Topic: F.03. Neuroendocrine Processes

Support: Grant CB-104264 Conacyt Mexico

Grant CB-169861 Conacyt Mexico

Title: Effect of ghrelin and leptin on voltage gated calcium channels in rin-m5f cells

Authors: *B. DOMINGUEZ MANCERA[1], A. HERNÁNDEZ-BELTRÁN[2], M. BARRIENTOS-MORALES[3], P. CERVANTES-ACOSTA[4,5], A. RODRÍGUEZ-ANDRADE[5]; [2]Lab. of Functional Alterations, [3]Lab. of Reproduction Biol., [4]Lab. of Mol. Biol., [1]Univ. Veracruzana, Dept Fisiología, Veracruz, Veracruz., Mexico; [5]Dept. of Chem. and Biochem., Inst. Tecnológico de Veracruz, Veracruz, Mexico

Abstract: The beta-pancreatic cells are in charge of producing and secreting insulin. These cells are electrically excitable and capable of generating action potentials (AP) spontaneously or in response to a stimulus. This electrical activity correlates with the secretory activity and is

orchestrated by ion channels. As a consequence, metabolic signals that modify the functional expression of these channels will alter the secretion pattern. Two hormones that regulate the voluntary food intake are Ghrelin (Ghr) and Leptin (Lep) by increasing or decreasing plasma insulin release alter glucose concentrations. In this context, the study of the electrophysiological mechanisms in beta-pancreatic cells associated to the effects of Ghr/Lep may shed light on the molecular processes involved in the endocrine/metabolic balance. In this work, we used the clonal insulin-producing RIN-M5f cell line as a model system and studied the effects of the chronic treatment of Ghr and Lep on the functional expression of voltage gated Ca^{2+} channels. Cell were kept in culture for 5 days in RPMI-1640 medium, supplemented with FBS 10%, L-glutamine (2%) and 1% penicillin-streptomycin and subjected to electrophysiological recording using the patch-clamp technique in the whole cell configuration in its two versions: current clamp and voltage clamp to dissect out the components of the Ca^{2+} current. In control conditions, the RIN-M5f cells displayed a pattern consisting of two states, silent and active. Interestingly Lep (10 nM, 72 h) significantly increased the frequency of the spontaneous APs and affected the morphology of the cells. Likewise, both Lep and Ghr treatment (10 nM, 72 h) significantly increased calcium current density from 13.2 ± 1.4 pA/pF in control cells, to 19.4 ± 2.8 and 25.2 ± 6.3 after Ghr and Lep treatment, respectively. Time course with Ghr 10 nM [0, 24, 48 and 72 h] showed increased currents as a function of exposure time. The analysis of the components of the calcium current showed that Ghr and Lep increased both LVA and HVA Ca^{2+} channels compared to the control. Hence, Ghr and Lep caused a 5- and 2.7-fold increase; respectively, in current amplitude through LVA channels. Likewise, Ghr and Lep treatment caused an increase in current amplitude of about 0.6- and 3.2-fold respectively, in current amplitude through HVA channels. Taken together these results suggest that Ghr or Lep long-term exposure increases calcium current density in RIN-M5f cells. It would be interesting how the changes in Ca^{2+} channel functional expression are related with the increase in electrical activity recorded in the cells after hormone treatment, and whether these changes in conjunction affect insulin release.

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Poster

398. Ca2+ Channels and Ca2+ Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 398.13/F8

Topic: B.10. Network Interactions

Support: Graduate School of Biomedical Sciences

Title: Differential translocation *In vitro* and *In vivo* of two closely related Neuronal Calcium Sensor Proteins Neurocalcin delta and Hippocalcin

Authors: *J. ZHANG[1], J. VIVIANO[1], A. KRISHNAN[2], P. BELAN[3], V. VENKATARAMAN[2]; [1]Grad. Sch. of Biomed. Sci., [2]Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [3]Bogomoletz Inst. of Physiol., Kiev, Ukraine

Abstract: Neuronal Calcium Sensor (NCS) proteins play a crucial role in mediating calcium signaling, which is critical for neuronal functions. The calcium-binding motif in these proteins are the EF hands. The calcium-myristoyl switch, which promotes calcium-dependent movement of the proteins to the membranes is another characteristic feature of this group of proteins. All proteins of this family are myristoylated at the N-terminus. Two NCS proteins, Hippocalcin (HPCA) and Neurocalcin delta (NCALD), share 88% identity and 95% similarity with each other. Yet they are non-redundant as shown by data from our and other laboratories. In order to investigate the differences in their properties, especially their response to changing calcium, two different approaches were used. For *in vitro* studies, bacterially expressed and purified proteins were assayed for their ability to bind to isolated membrane fractions in a calcium-dependent fashion. For *in vivo* studies, YFP-tagged NCALD and HPCA constructs were independently transfected into COS7 cells and calcium-dependent translocation was monitored. The results demonstrate significant differences in translocation between NCALD and HPCA.

Disclosures: J. Zhang: None. **J. Viviano:** None. **A. Krishnan:** None. **P. Belan:** None. **V. Venkataraman:** None.

Poster

399. Dopamine Transporter Regulation

Location: Halls B-H

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Program#/Poster#: 399.01/F9

Topic: B.05. Transporters

Support: NIH Grant DA15169 to HEM

NIH Fellowship DA039592 to CGS

Title: Dopamine transporter amino and carboxy termini synergistically mediate ack1-dependent endocytosis

Authors: *C. G. SWEENEY, B. P. TREMBLAY, H. E. MELIKIAN;
Brudnick Neuropsychiatric Res. Inst., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Dopamine (DA) signaling is central to movement, cognition, motivation, and reward. Following vesicular release, extracellular DA levels are temporally and spatially constrained by presynaptic reuptake, mediated by the dopamine transporter (DAT). DAT surface expression is acutely modulated by psychostimulants, such as cocaine and amphetamine, and by cellular signaling proteins, such as protein kinase C (PKC). Under basal conditions, DAT traffics from and to the plasma membrane via endocytosis and recycling, and an Ack1-mediated negative regulatory endocytic mechanism (endocytic brake) stabilizes DAT at the plasma membrane. PKC inactivates Ack1, releases the endocytic brake, and stimulates DAT, but not serotonin transporter (SERT), internalization. Reports from our laboratory and others independently implicate both DAT intracellular N- and C-termini as required for the endocytic brake. However, whether 1) Ack1 associates with DAT intracellular domains and 2) whether an independent braking mechanism exists at both N- and C-termini or one synergistic mechanism requiring both domains remains unknown. To test whether DAT N- and C-termini work synergistically to impose the endocytic brake, we generated DAT/SERT chimeras in which we replaced DAT N-, C-, or both termini with those of SERT. We made the reciprocal substitutions on SERT, substituting intracellular DAT domains. DAT and SERT are homologous transporters, yet they share little sequence identity at their N- and C-termini. They both undergo PKC-stimulated endocytosis, but only DAT requires Ack1 inhibition for this process. This finding poses Ack1 as a potential candidate for mediating the hypothesized synergy between DAT's N-and C-termini. We first tested that each of the DAT/SERT chimeras functions properly via [^{3}H]DA or [^{3}H]5HT uptake kinetics. Next we tested via internalization assay whether DAT N- and C-termini are required to release the endocytic brake in response to Ack1 inhibition with AIM-100. Substituting SERT's N- or C- termini alone onto DAT did not affect AIM-100 stimulated endocytic rates; however, placing both SERT domains on DAT abolished Ack1-mediated endocytic braking. These findings suggest that while, individually, each DAT terminus is not required independently for the brake, they may synergistically facilitate the braking scaffold. Tests using SERT chimeras expressing DAT N- and C-termini will address the sufficiency of these domains in this process. Future studies will also test whether Ack1 and DAT associate via N- and/or C-terminal-dependent processes.

Disclosures: **C.G. Sweeney:** None. **B.P. Tremblay:** None. **H.E. Melikian:** None.

Poster

399. Dopamine Transporter Regulation

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 399.02/F10

Topic: B.05. Transporters

Support: NIH Grant DA15169 to HEM

Title: Using *Drosophila melanogaster* as a model to study how regulated dopamine transporter trafficking impacts psychostimulant associated reward

Authors: *R. R. FAGAN[1], P. EMERY[2], H. E. MELIKIAN[3];
[2]Neurobio., [3]Brudnick Neuropsychiatric Res. Inst., [1]Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: The neurotransmitter dopamine (DA) is essential for movement, cognition and reward. The plasma membrane dopamine transporter (DAT) spatially and temporally limits DA neurotransmission in order to terminate signaling and replenish presynaptic DA stores. Recent reports have identified DAT coding variants that underlie a variety of DA-related neuropsychiatric conditions, including attention-deficit/hyperactivity, autism spectrum and bipolar disorders. Thus, DA-related behaviors are highly sensitive to intact DAT function. Addictive psychostimulants, such as cocaine and amphetamine, are competitive DAT inhibitors and substrates, respectively, that perturb DA reuptake and increase extracellular DA concentrations. In addition to pharmacological inhibition, DAT activity is acutely diminished in response to protein kinase C (PKC) activation and amphetamine exposure via dynamic endocytic trafficking. Studies from our lab and others have demonstrated that numerous proteins, including the small, neuronal GTPase, Rin (RIT2) and the nonreceptor tyrosine kinase, Ack1, are critical factors in controlling DAT surface stability and endocytic response to PKC stimulation. However, it is unknown whether these regulators of DAT endocytosis play a requisite role in establishing DA-related behaviors such as reward. *Drosophila melanogaster* is a powerful tool for studying the consequences of altered DA homeostasis and DAT function *in vivo*. In particular, 3rd instar *Drosophila* larvae express ~120 DAergic neurons, and are a simple model system to study associative learning and reward behaviors *in vivo*. A recent report by Rohwedder and colleagues found that DA neurotransmission by the four primary protocerebral anterior medial (pPAM) neurons is both necessary and sufficient for odor-sugar associative reward. Here we show that wild-type *Drosophila* larvae also exhibit significant odor-amphetamine associative reward. The simple and efficient design of this behavioral paradigm will facilitate further exploration of the role of *Drosophila* Rin and Ack1 homologues in regulated DAT surface expression and rewarding behaviors.

Disclosures: R.R. Fagan: None. **P. Emery:** None. **H.E. Melikian:** None.

Poster

399. Dopamine Transporter Regulation

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 399.03/F11

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA038598

Title: Role of G protein betagamma subunits in amphetamine-stimulated increase in extracellular dopamine

Authors: *S. S. HARRIS[1], M. TERMINEL[2], E. CASTANEDA[3], J. C. MAUNA[4], E. THIELS[4], G. E. TORRES[1];
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Abstract: The dopamine transporter (DAT) plays a role in several psychiatric disorders, including ADHD, narcolepsy and drug addiction. DAT is also a molecular target for amphetamines, used in the treatment of both ADHD and narcolepsy. However, due to their rewarding and addictive properties, amphetamine use can lead to abuse and addiction. Amphetamines increase synaptic levels of DA by binding to DAT and causing efflux DA through DAT. The mechanism by which amphetamines induces DA efflux is not understood. Therefore, it is important to identify the molecular events involved in the actions of amphetamines. Our lab has identified a novel interaction between G protein βγ subunits and DAT that plays a role in DAT function. Our *in vitro* studies, using cell systems, show that activation of Gβγ subunits with the Gβγ activator mSIRK promotes DA efflux through DAT, similar to the actions of amphetamine and inhibition of Gβγ subunits by the Gβγ inhibitor gallein decreases amphetamine-induced DA efflux. Moreover, findings from our *in vivo* studies show that inhibition of Gβγ activation by both systemic gallein administration and local infusion of gallein into the nucleus accumbens, a brain region important for both the rewarding and locomotor activating effects of amphetamine, results in an attenuation of amphetamine-induced increase in locomotor activity in rats. The proposed study sought to determine whether a potential mechanism for the effects of Gβγ inhibition on amphetamine-induced locomotor activity is the involvement of Gβγ subunits in amphetamine-induced increase in extracellular DA levels in the nucleus accumbens using *in vivo* microdialysis. Rats underwent stereotaxic surgery to implant a guide cannula into the nucleus accumbens. Following recovery from surgery, the animals were implanted with a microdialysis probe through the guide cannula and extracellular DA levels were assessed by HPLC with electrochemical detection. DA levels were monitored prior to treatment of the animals with gallein and/or amphetamine. The animals were first

administered gallein (or vehicle) and 30 minutes later amphetamine (or vehicle) and extracellular DA levels continued to be monitored following amphetamine treatment. Our preliminary results show that inhibition of Gβγ activation by gallein results in attenuation of amphetamine-induced extracellular DA levels in the nucleus accumbens. The result suggests that the Gβγ-DAT interaction plays a role in both amphetamine-induced increases in extracellular DA levels and locomotor activation. The results of the present study expand our current understanding of the molecular mechanisms responsible for amphetamine's actions in the brain.

Disclosures: S.S. Harris: None. **M. Terminel:** None. **E. Castaneda:** None. **J.C. Mauna:** None. **E. Thiels:** None. **G.E. Torres:** None.

Poster

399. Dopamine Transporter Regulation

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Topic: B.05. Transporters

Support: NIH Award MH095044 (RDB)

MH093102 (JAH)

Title: Presynaptic determinants of dopamine signaling *In vivo* elucidated via forward genetic analysis of swimming induced paralysis (Swip)

Authors: *O. REFAI[1], J. HARDAWAY[3], C. L. SNARRENBERG[3], S. ROBINSON[4], S. L. HARDIE[3], P. FREEMAN[4], R. D. BLAKELY[2];
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Abstract: Dopamine (DA) is a phylogenetically conserved neurotransmitter that regulates a variety of complex behaviors. The presynaptic, DA transporter (DAT) acts to limit DA action at synaptic and extra-synaptic DA receptors. In humans, mutations impacting DAT protein expression or structure are associated with multiple brain disorders including schizophrenia, juvenile Parkinsonism/dystonia, and addiction. Loss of function mutations in the nematode *C. elegans dat-1* gene result in a phenotype termed Swimming-induced paralysis (Swip). The Swip phenotype of *dat-1* mutants can be rescued by either treatment of animals with the vesicular monoamine transporter (*cat-1*) inhibitor reserpine or by a genetic cross to animals lacking expression of the TH ortholog CAT-2, or the D2-type DA receptor DOP-3. In a forward genetic

screen for determinants of DA signaling, our lab isolated multiple, novel mutations that affect DA signaling, including alleles of *dat-1*. Here we describe our efforts to characterize two mutant lines, *vt39* and *vt44*, using genetic and pharmacological approaches. Direct genomic sequencing, and complementation tests, indicate that both lines harbor a normal *dat-1* gene. Using a single nucleotide polymorphism-based mapping approach, we find that *vt39* and *vt44* map to chromosomes I and III, respectively. Reserpine treatment rescued the Swip phenotype of *vt39* and *vt44,* similar to *dat-1* mutants, suggesting that they function in the same pathway as *dat-1*. To examine this hypothesis, *vt39* and *vt44* lines were crossed to *cat-2* or *dop-3* mutants. Here again, loss of function mutations in *cat-2* or *dop-3* rescued the Swip phenotype of *vt39* and *vt44.* Similar to *dat-1* mutants*, vt39 and vt44* exhibited osmo-sensitivity, as shown by suppression of their Swip phenotype in M9 buffer or 150mOsm sucrose medium. Since *dat-1* single mutant exhibit a highly penetrant Swip phenotype, swimming assays for *dat-1;vt39* or *dat1;vt34* double mutants may not allow for dramatic additivity. To pursue contributions of DAT-linked pathways more rigorously, we treated *vt39* and *vt44* mutants with the DAT-1 inhibitor nisoxetine, which we have shown to trigger Swip in wildtype animals within minutes of incubation. Using this reagent, we found that *vt39* and *vt44* mutants in both water and 150mOsm conditions, after treatment with 20 uM nisoxetine, revealed no significant additive effect as compared to *dat-1* animals, suggesting that the genes harbored by these mutations act in the same pathway as DAT-1. Further studies related to the identity of the genes encoding the *vt39* and *vt4*4 mutations and their functional analyses will be presented. Supported by NIH Award MH095044 (RDB) and MH093102 (JAH).

Disclosures: O. Refai: None. **J. Hardaway:** None. **C.L. Snarrenberg:** None. **S. Robinson:** None. **S.L. Hardie:** None. **P. Freeman:** None. **R.D. Blakely:** None.

Poster

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R01NS071122-A1

S10OD020026

Title: Methamphetamine regulates the firing activity of dopamine neurons via a calcium-dependent potassium channel

Authors: M. LIN, D. SAMBO, *H. KHOSHBOUEI;
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Abstract: Methamphetamine (METH) is a highly addictive psychostimulant. METH targets biogenic amine transporters leading to increased levels of biogenic amines in the CNS and periphery. The rewarding effects of METH are primarily due to its ability to increase dopamine levels in the brain. While METH has been shown to alter the excitability of dopamine neurons, the mechanism of METH regulation of the intrinsic firing behaviors of dopamine neurons are less understood. In this study, we identified an unexpected and unique property of METH on the regulation of firing activity of dopamine neurons. We found that although METH produced a transient augmentation (30 sec - 3 min) of the spontaneous spike activity of midbrain dopamine neurons, this was followed by a progressive reduction of the spontaneous spike activity. Careful inspection of action potentials revealed METH treated neurons exhibited increased half-width and larger coefficients of variation of the interspike interval relative to baseline, suggesting METH exposure may affect the activity of voltage-dependent potassium channels in these neurons. The unexpected finding that METH broadened the action potential and decreased the amplitude of afterhyperpolarization (AHP) led us to ask whether these two outcomes are interrelated or independent. Using excised patch single-channel recordings, first we identified BK channels in dopamine neurons by their voltage-dependence and their responses to a BK channel blocker or opener. We found while METH suppressed the amplitude of BK-mediated unitary currents, the BK channel opener NS1619 attenuated the effects of METH on action potential broadening, AHP repression, and spontaneous spike activity reduction. Live cell TIRF microscopy, electrophysiology and biochemical analysis data suggest METH exposure decreased the activity of BK channels by decreasing the level of BK-α subunits at the plasma membrane. Taken together, these data suggest BK channels may regulate the excitability of dopamine neurons and that METH regulation of the intrinsic firing behaviors of dopamine neurons is, in part, due regulation of BK channels.

Disclosures: **M. Lin:** None. **D. Sambo:** None. **H. Khoshbouei:** None.

Poster

399. Dopamine Transporter Regulation

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Support: R01DA026947-A1

R01NS071122-A1

S10OD020026

Title: The sigma-1 receptor decreases methamphetamine stimulation of the dopamine transporter via a calcium-dependent mechanism

Authors: *D. O. SAMBO, M. LIN, H. KHOSHBOUEI;
Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: The dopamine (DA) transporter (DAT) is a transmembrane protein implicated in multiple physiological and pathological conditions, including movement, reward, neurodegeneration, and drug addiction. DAT functions to reuptake DA from the extracellular space, making it a crucial regulator of DA homeostasis. DAT is also the primary target for methamphetamine (METH), a highly addictive psychostimulant. METH increases extracellular DA levels through interactions with DAT via at least three well-characterized mechanisms: 1) decrease in DA uptake, 2) induction of DA efflux, and 3) internalization of the transporter. Additionally, our lab and others have shown that METH increases the firing activity of DA neurons in a DAT-dependent manner. Through these mechanisms, METH has been shown to increase extracellular DA levels up to 1000% in the brain, leading to both its rewarding effects and high abuse potential. While there are currently no treatments for METH addiction, a number of studies have indicated that a protein called the sigma-1 receptor (σ_1R) is a promising target for METH addiction, with a number of studies demonstrating the efficacy of σ_1R ligands in decreasing the effects of METH. The σ_1R is a neuroprotective chaperone protein that can be activated by selective ligands. In this study, we investigated the potential mechanisms by which the σ_1R regulates METH-mediated DAT activity. We found that treatment with the selective σ_1R agonist PRE-084 prevented METH-induced, DAT-mediated increases in firing activity in dopaminergic neurons and also decreased METH-mediated DA efflux, without affecting DA uptake or DAT trafficking. Consist with these findings, we found that treatment with PRE-084 decreases METH-induced locomotor activity in rodents. To investigate the effects of σ_1R on the rewarding properties of METH, we used conditioned place preference (CPP) as a model of drug additive behavior. We found that PRE-084 treatment reduces the reinstatement of METH CPP. Currently, we are investigating the potential molecular mechanisms by which the σ_1R reduces the effects of METH. We found that the σ_1R interacts with the DAT, thus potentially influences its activity. Importantly, we showed that σ_1R activation by PRE-084 substantially reduced METH-stimulated, DAT-dependent increases in intracellular calcium levels. This is consistent with our findings that σ_1R activation decreases both METH-stimulated DA efflux and firing activity, which are both modulated by METH-stimulated calcium increases. Overall, these findings suggest that σ_1R activation may reduce the effects of METH through modulation of DAT activity and similarly decreases METH-induced behaviors.

Disclosures: D.O. Sambo: None. **M. Lin:** None. **H. Khoshbouei:** None.

Poster

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Topic: B.05. Transporters

Support: NIH grant MH106912

Title: Mechanistic and behavioral characterization of the dopamine transporter using a novel allosteric modulator

Authors: *S. AGGARWAL[1], P. MENELL[2], A. CHANG[2], S. KORTAGERE[3], O. V. MORTENSEN[2];
[1]Pharmacol. & Physiol., Drexel Univ., Philadelphia, PA; [2]Dept. of Pharmacol. & Physiol., [3]Dept. of Microbiology & Immunol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The dopamine transporter (DAT) serves a pivotal role in controlling dopamine (DA)-mediated neurotransmission by clearing DA from synaptic and perisynaptic spaces, and controlling its action at postsynaptic DA receptors. Major drugs of abuse such as amphetamine and cocaine interact with DAT to mediate their effects by enhancing extracellular DA concentrations. Thus, DAT is a viable target for the treatment of psychostimulant abuse and addiction. We have recently identified a novel allosteric site that lies outside the central substrate and inhibitor binding pocket of DAT. This allosteric site was identified using information derived from comparative modeling of human and parasite *Schistosoma mansoni* monoamine transporters. Site-directed mutagenesis has validated the functional significance of this allosteric site. In addition, the hybrid structure–based (HSB) method has identified conformation-specific molecules that can potentially stabilize the transporter in certain beneficial conformations that may alter its interaction with cocaine and amphetamine without affecting uptake activity of DA. Interestingly, KM822, one of the ligands identified in a virtual screening experiment, was found to decrease the affinity of cocaine for DAT by 3-folds in an *in vitro* cell-based assay. In addition, KM822 also reduced the potency of cocaine towards DAT-mediated DA reuptake inhibition in an *ex vivo* model of striatal synaptosome preparations. The preliminary *in vivo* effects of KM822 on cocaine potency were tested on psychostimulant-associated behaviors in a planarian model where KM822 specifically inhibited the locomotion elicited by DAT-interacting stimulants amphetamine and cocaine. To further identify the structural determinants of KM822 allosteric binding site, we are employing substituted cysteine scanning accessibility methods (SCAM) and biotinylation experiments to identify residues that directly interact with KM822. Additional studies will be employed to determine the proximity of KM822 binding domain with the extracellular gate formed by Arg85-Asp476 salt bridge in hDAT. These studies will provide details on the role of Arg85-Asp476 extracellular gate in KM822-mediated modulation of DAT

interaction with its substrates and inhibitors. Overall, KM822 provides a unique opportunity as a molecular probe to identify novel mechanistic aspects of DAT function. In addition, it can lead to the development of more unique compounds with promising potential to treat drug addiction.

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Poster

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R01NS071122-A1

S10OD020026

Title: Kv2.1 and the dopamine transporter interact in dopaminergic neurons

Authors: ***J. LEBOWITZ**[1], J. A. PINO REYES[2], S. STREIT[3], D. SAMBO[1], M. LIN[1], H. KHOSHBOUEI[1], G. E. TORRES[2];
[1]Neurosci., [2]Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL; [3]Col. of Medicine, Heinrich-Heine-Universität, Düsseldorf, Germany

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder that, by current estimates, is present in as many as one million Americans. A key element of PD pathology is the loss of dopaminergic neurons of the substantia nigra (SNc). The cellular mechanism for the apparent susceptibility of SNc dopamine neurons to neurodegeneration remains enigmatic. Differences in the tonic firing rate, inherent excitability, and intracellular Ca^{2+} buffering capacity in the SNc dopamine neurons are thought to contribute to the selective loss seen in PD. The dopamine transporter (DAT) regulates the dimension and duration of dopamine signaling, and is implicated in PD. Kv2.1 is a high-threshold voltage gated potassium channel that is responsible for controlling neuronal repolarization and excitability in nearly all neuronal subtypes. Since DAT carries an inward depolarizing current, and its activation increases neuronal excitability, we tested the hypothesis that a functional interaction between Kv2.1 and DAT contributes to the selective vulnerability of SNc dopamine neurons. This hypothesis in supported by our preliminary data that Kv2.1 and DAT exist in a protein complex as detected by their co-immunoprecipitation in the striatum and midbrain, two DAT-rich brain regions. In addition, we

found that all dopaminergic neurons in the SNc and ventral tegmental area (VTA) invariably express Kv2.1. The degree of Kv2.1 immunolabeling was similar in both brain regions. Membrane Kv2.1 exists in both non-conducting clusters and conductive non-clustered forms. Channel declustering is coupled with a hyperpolarization of activation voltage, presumably to protect neurons from excitotoxicity. Declustering of Kv2.1 complexes has been shown to be mediated by intracellular Ca^{2+} release via the activation of calcineurin. Previous work by our group has also shown that DAT activation by methamphetamine increases intracellular Ca^{2+}. While our data strongly support the idea that Kv2.1 is expressed at the membrane of dopamine neurons and it is potentially colocalized with DAT, whether DAT activation affects the ratio of conductive/non-conductive channels is not known. Ongoing high-resolution imaging and electrophysiological experiments will examine Kv2.1 activity in the SNc and VTA dopamine neurons at resting condition and following DAT activation. The results will reveal novel cellular pathways contributing to PD.

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Poster

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NIH award MH086530 (to R.D.B.)

NRSA award MH107132-02 (G.L.D.)

Title: Altered sensitivity to serotonin transporter blockade underlies loss of locomotor response to cocaine in DAT Val559 mice

Authors: *A. STEWART[1,2], G. L. DAVIS[1,2,3], R. GOWRISHANKAR[3,1,2,4], P. J. GRESCH[1,2], F. I. CARROLL[5], M. K. HAHN[1,2], R. D. BLAKELY[1,2];
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Abstract: Pathological alterations in dopamine (DA) homeostasis have been implicated in multiple neuropsychiatric disorders including Attention-Deficit/Hyperactivity Disorder (ADHD).

In an effort to identify penetrant genetic changes in DA signaling that drive ADHD risk and generate improved, construct-valid animal models of ADHD, the Blakely lab screened for rare coding variation in the DA transporter (DAT, *SLC6A3*) in ADHD subjects, identifying the Ala559Val substitution. *In vitro* studies revealed that the Val559 substitution promotes spontaneous DA efflux that can be antagonized by both methylphenidate (MPH) and amphetamine (AMPH), commonly prescribed ADHD medications. Previous work has shown that mice harboring the DATVal559 mutation display tonically-elevated extracellular DA levels as well as reductions in both AMPH-induced DA release and AMPH-induced locomotor stimulation. Here we report that, in contrast to findings with AMPH and MPH, the locomotor-stimulating actions of cocaine (COC) are completely absent in DAT Val559 mice, even at a high drug dose (30 mg/kg). Systemic COC-evoked DA elevations in the dorsal striatum are also lost in DAT Val559 mice. In the context of prior studies revealing normal DA D1 receptor agonist-induced locomotor activity, these studies indicate that key perturbations in DA signaling derive from cell-autonomous alterations in DA neuron physiology and activation. Given that one predominant difference between COC and AMPH/MPH is a high affinity interaction with the presynaptic 5-HT transporter (SERT), we hypothesized that the DAT Val559 allele induces compensations in 5-HT signaling that can inhibit locomotor activation by COC. Supporting this idea, combined inhibition of SERT and DAT through concurrent administration of the SERT blocker fluoxetine completely abolished MPH-induced locomotor activity, mimicking the actions of COC, in DAT Val559 mice. Additionally, injection of animals with the COC analog (RTI-113), which exhibits comparatively reduced action at SERT versus COC, induces dose-dependent locomotor activation in DAT Val559 mice, more closely resembling the activities of AMPH and MPH. These data support the hypothesis that the lack of COC-induced hyperlocomotion in DAT Val559 mice results from the combined high affinity targeting of DAT and SERT by COC and suggests that penetrant serotonergic plasticities have arisen in the DATVal559 model that can override DA-driven locomotor activation. Ongoing efforts seek to complement these studies through a genetic approach where the DATVal559 allele is expressed in the background of SERTMet172, a variant that demonstrates reduced sensitivity to COC.

Disclosures: **A. Stewart:** None. **G.L. Davis:** None. **R. Gowrishankar:** None. **P.J. Gresch:** None. **F.I. Carroll:** None. **M.K. Hahn:** None. **R.D. Blakely:** None.

Poster

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Support: Intramural Research Program NIMH

Title: N-terminus phosphorylation in the Dopamine Transporter mediates Gβγ-stimulated dopamine efflux

Authors: *J. GARCIA-OLIVARES, J. A. BORIS, S. G. AMARA;
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Abstract: Monoaminergic neurotransmission (dopamine, serotonin, and norepinephrine) is altered in complex psychiatric conditions such as depression, attention-deficit hyperactivity disorder (ADHD) and drug addiction. For dopamine, the dopamine transporter (DAT) clears extracellular dopamine through a sodium-coupled transport mechanism. The function of DAT is regulated by many intracellular mechanisms including phosphorylation, ubiquitination, and protein-protein interactions. We recently reported a novel mechanism for the regulation of DAT by heterotrimeric G proteins. We found that Gβγ subunits bind directly to the C-terminus of DAT and, upon G protein activation, the release of Gβγ results in a decrease in DA uptake. In a new set of studies, it was found that the decrease in DA uptake is a result of the promotion of DA efflux, an effect that may be similar to the DA efflux produced by amphetamines. DA efflux is dependent on calcium, sodium, and membrane potential. It also involves phosphorylation of the N-terminus by Serine/Threonine kinases such as protein kinase C (PKC) and calmodulin kinase II (CamKII). Using radiolabeled DA to measure DAT function, we are now exploring whether the DA efflux promoted by the activation of Gβγ subunits also requires phosphorylation of the N-terminus. We used two DAT mutants, hDAT-S/A and hDAT-S/D. One mutant has the five N-terminal serines (S2, S4, S7, S12, S13) substituted with alanine (S/A) to eliminate phosphorylation and the other mutant contains aspartate residues at these sites (S/D) to mimic a phosphorylated state. The induction of [3H]-DA efflux by mSIRK, a peptide that binds and activates Gβγ, was abolished in the mutant carrier hDAT-S/A, suggesting that the serine residues are important for the Gβγ-stimulated efflux. We also used pharmacological tools to inhibit kinases and phosphatases to explore how the general phosphorylation state of DAT is important in the regulation of DAT by Gβγ subunits. Our data suggest that the effect on uptake and efflux mediated by Gβγ activation is dependent on the availability of phosphorylation sites in the DAT N-terminus. Our current efforts aim to establish whether the substitutions of putative phosphorylation sites modify the binding of Gβγ to the C-terminus and whether phosphorylation is required for the conformational transitions that shift the transporter into an efflux mode.

Disclosures: J. Garcia-Olivares: None. **J.A. Boris:** None. **S.G. Amara:** None.

Poster

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NIH grant RR0254970

Title: Dopamine transporter interactome when exposed to psychostimulants

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Abstract: The dopamine transporter (DAT) is a neurotransmitter transporter essential for the reuptake of released dopamine (DA) in the brain. DAT is one of the main targets for psychostimulants leading to a disruption of DA homeostasis. To begin to understand what proteins regulate this process, we used tandem mass spectrometry to examine the interacting proteome of DAT when exposed to methamphetamine, a DAT substrate, and cocaine, a DAT blocker. Here we provide a comprehensive qualitative analysis of proteins detected in response to psychostimulant treatment. Already known interacting proteins such as PKA, PKC, and alpha-synuclein were detected with variability based on treatment. Post spectroscopy analysis revealed novel protein interactions including redox, actin-binding, calcium binding/regulating, and voltage-dependent proteins based on drug treatment. Our MS data provides evidence that differential binding proteins are involved in DAT-mediated drug response.

Disclosures: S. Ingram: None. **T. Rana:** None. **J.S. Goodwin:** None.

Poster

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Support: MH105094

MH107132-02

Title: Aberrant dopamine D2 autoreceptor regulation of the dopamine transporter in striatal dopaminergic terminals of mice expressing the ADHD-associated dopamine transporter variant DAT Val559

Authors: *R. GOWRISHANKAR[1,2,3], G. L. DAVIS[1,2,3], A. M. STEWART[1,2], J. S. RIELE[4], M. K. HAHN[1,2], R. D. BLAKELY[1,2];
[1]Dept. of Biomed. Sciences, Charles. E. Schmidt Col. of Med., Florida Atlantic Univ., Jupiter, TN; [2]Brain Inst., Florida Atlantic Univ., Jupiter, FL; [3]Neurosci. Grad. Program, [4]Neurosci. Undergraduate Program, Vanderbilt Univ., Nashville, TN

Abstract: Dopamine (DA) D2 autoreceptors (D2ARs) play a central role in modulating DA signaling at DA terminals by reducing DA synthesis, by inhibiting DA vesicular release and by enhancing the surface availability of the presynaptic DA transporter (DAT), although the details of the latter interaction in vivo are poorly defined. DAT is responsible for terminating DA signaling at the synapse and is the target for the most common pharmacological therapies for Attention-Deficit/Hyperactivity Disorder (ADHD), amphetamine (AMPH) and methylphenidate (MPH). We previously reported that mice expressing the ADHD-associated DAT variant Val559 exhibit pronounced elevations in extracellular DA and decreased depolarization-induced vesicular DA release from DA terminals in the striatum. Moreover, we demonstrated that reduced vesicular DA release was sustained by high tonic DA stimulation of presynaptic D2ARs. Here we report that DAT Val559 mice exhibit a loss of D2AR-dependent tyrosine hydroxylase (TH) phosphorylation at Ser40, a function shown previously to be important for D2AR regulation of DA signaling. In order to understand how elevated DAergic tone affects D2AR regulation of DAT, we pursued slice biotinylation studies, where we could demonstrate a basal elevation of striatal DAT surface protein levels that, unlike wildtype DAT, cannot to be further enhanced by D2AR stimulation with quinpirole. Finally, we probed striatal slices for evidence of altered DAT phosphorylation at Val559, a site previously demonstrated to support DAT-mediated DA efflux in response to AMPH. Here, we demonstrated elevated basal Thr53 phosphorylation in DATVal559 slices along with an inability of D2AR stimulation to enhance phosphorylation, whereas slices from wildtype animals support D2AR elevations in Thr53 phosphorylation. Collectively, our data raise the possibility that aberrant presynaptic D2AR

signaling, observed either in DA synthesis capacity, DA release or DA clearance, may contribute to the pathophysiology of ADHD.

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Poster

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Topic: B.05. Transporters

Support: P3E UDG 2015

Title: Dopamine transporter expression evaluated in perinatally asphyxiated rats

Authors: *S. J. LOPEZ-PEREZ[1], J. U. MORA-VENADERO[2];
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Abstract: The perinatal asphyxia (PA) produces alterations in the mammal Nervous System, and it is related with the development of the specific neurochemistry conditions in the dopaminergic system, that could be associate with ADHD, schizophrenia and depression. The dopamine transporter (DAT) it is responsible to maintaining the normal extracellular levels of dopamine in prefrontal cortex and striatum, and decrements in its expression could be important in the generation of neurochemistry substrates of these pathologies. Previous evidence suggest that AP event should modify DAT expression, therefore, the objective of this work was to analyze the DAT expression in juvenile rats, which were asphyxiated perinatally. At 11 days old, female and male rats were asphyxiated for 45 min in a small sealed asphyxia chamber; when the animals reached 30 days old, were sacrificed to obtain the prefrontal cortex and striatum, regions which were homogenized in RIPPA buffer supplemented whit protease inhibitors. Homogenized were centrifuged to collect the supernatant and total content of proteins was estimated in it. 80 µg of proteins were used to make a Western blot and analyze quantitatively the levels of DAT expression, using the 3-3´-diaminobenzidine tetrahydrochloride. Results showed higher DAT expression level in frontal cortex and striatum of asphyxiathed animals, independently of sex. Previously, we demonstrated a low dopamine release in frontal cortex from 30 days old male rats, so the overexpression of DAT reported here may reflect a compensatory mechanism to uptake the dopamine that maybe is flowing far away of synapse, in view of the dopaminergic volume transmission fact. This work was partially supported by P3E UDG founding.

Disclosures: **S.J. Lopez-Perez:** None. **J.U. Mora-Venadero:** None.

Poster

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Title: Identification of residues involved in the dopamine transporter-G betagamma interaction and dopamine efflux

Authors: ***J. A. PINO**[1], M. H. CHENG[3], F. PULLARA[3], A. GOPINATH[2], K. SAHA[2], J. LEBOWITZ[2], H. KHOSHBOUEI[2], J. GARCIA-OLIVARES[4], S. G. AMARA[4], I. BAHAR[3], G. E. TORRES[1];
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Abstract: The dopamine transporter (DAT) plays a crucial role in the regulation of brain dopamine (DA) homeostasis. Through re-uptake of DA, DAT serves two important functions: the termination of synaptic transmission at dopaminergic terminals, and the replenishment of vesicular DA pools. In addition to uptake, DAT can also function to release DA. This process, which is referred to as DAT-mediated efflux, is the mechanism used by potent and highly addictive psychostimulants, such as amphetamine and its analogues, to increase extracellular DA levels in motivational and reward areas of the brain. It has long being recognized that DA neurons release DA through exocytotic and non-exocytotic processes. However, the exact mechanism by which physiological signals or psychostimulants, such as amphetamine, induce DA efflux through DAT still remains a complex and not completely understood area of research. Thus, examining the basic mechanism(s) that affect DA efflux through DAT is critical for both understanding fundamental aspects of DA regulation and clinical intervention in DA-related brain disorders associated with the therapeutic use and abuse of psychostimulants. Recently, we discovered that the βγ subunits of G protein (Gβγ) bind to the intracellular carboxy-terminus of DAT and regulate transporter activity. More importantly, we have observed that activation of Gβγ promotes DAT-mediated DA efflux. However, the amino acid residues involved in Gβγ interaction site(s) in DAT and their role in transporter regulation remain largely unknown. Here, we used a combination of computational modeling, mutagenesis, immunoprecipitations, and

functional assays to identify the Gβγ binding site on DAT. Based on the Drosophila melanogaster DAT crystal structure, we used advanced molecular modeling approaches to generate equilibrium conformations for human DAT (hDAT) in different states. Simulations of Gβγ binding to hDAT indicate that Gβγ has a high propensity for binding a hot spot within the intracellular carboxy-terminus of hDAT. The model predicts that R588 and R610 in hDAT are critical for interaction with Gβγ. Preliminary functional studies are consistent with the role of these residues in Gβγ interaction with DAT and promotion of DA efflux. Thus, this study provides a detailed characterization of the DAT-Gβγ interaction and a better understanding of its contribution to DAT-mediated efflux

Disclosures: **J.A. Pino:** None. **M.H. Cheng:** None. **F. Pullara:** None. **A. Gopinath:** None. **K. Saha:** None. **J. Lebowitz:** None. **H. Khoshbouei:** None. **J. Garcia-Olivares:** None. **S.G. Amara:** None. **I. Bahar:** None. **G.E. Torres:** None.

Poster

399. Dopamine Transporter Regulation

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: B.05. Transporters

Support: MH107132-02

MH105094

Title: Changes in motivation, impulsivity, cognition and DAergic signaling cascades with ADHD-associated DAT Val559 transgenic mouse.

Authors: *G. L. DAVIS[1,2,3], L. A. WALKER[4], R. D. BLAKELY[1,2];
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Abstract: Our lab has created a construct-valid mouse model of ADHD based on the dopamine (DA) transporter (DAT) coding variant Val559 to elucidate neurobiological mechanisms supporting Attention-Deficit hyperactivity Disorder (ADHD) and other DA-linked, comorbid disorders. Previously we demonstrated that DAT Val559 mice have altered behavioral response to amphetamine (AMPH), postulating that it was through a constitutively active D2 receptor (D2R)-DAT interaction that resulted in reduced vesicular DA release. We have further explored this altered drug response by examining downstream signaling proteins that are known to play a

role in AMPH-induced locomotor behavior, such as ERK1/2, as well as changes in cellular activation, and the possibility of rescuing effects via D2R-dependent mechanisms. Our preliminary findings indicate that downstream pathways exhibit a blunted response, mirroring the behavior. Additionally, we have sought evidence for alterations in cognitive, attention and impulsivity using the 5-choice serial reaction time task (5-CSRTT), and used progressive ratio and sucrose preference tasks to examine changes in motivation and hedonic valuation. Alterations in cognitive performance, motivation, and impulsivity were observed in the DAT Val559 mice, with no apparent changes in hedonia. DAT Val559 mice showed several key differences in the 5-CSRTT task, including faster acquisition of the task, increased impulsivity under a long delay condition, yet improved performance with a variable delay condition. Interestingly, altered impulsive responses in the context of the timing parameter of the paradigm suggests that DATVal559 mice may exhibit an alteration in timing perception. Ongoing studies seek to clarify this effect and to determine whether this alteration contributes to the increased impulsivity seen in 5-CSRTT. Additionally, the DATVal559 mice displayed increased break points in the progressive ratio task. This alteration in apparent motivation could drive the DATVal559 to hyperfocus on the parameters driving task acquisition in the 5-CSRTT and to differentially utilize the temporal information presented during training sessions, being less reliant on absolute timing expectations. Ongoing studies seek to determine the cellular and circuit level plasticities that derive from lifelong expression of the DAT Val559 variant and that lead to the observed changes in impulsivity and motivation, and determine how these related to alterations observed in AMPH and methylphenidate drug responses. Supported by NIH Awards MH107132-02 (GLD) MH105094 (RDB).

Disclosures: **G.L. Davis:** None. **L.A. Walker:** None. **R.D. Blakely:** None.

Poster

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Topic: B.05. Transporters

Support: R01DA026947-A1

R01NS071122-A1

S10OD020026

Title: Noncanonical neurotoxicity of hiv-1 tat on midbrain dopamine neurons

Authors: *D. MILLER[1], S. STREIT[2], K. SAHA[1], S. BUCH[3], W. STREIT[1], J. MCLAUGHLIN[1], H. KHOSHBOUEI[1];
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Abstract: The transactivator of transcription protein, HIV-1 Tat, is linked to HIV-associated neurocognitive disorder (HAND). HIV-1 Tat is produced in $CD4^+$ cells such as microglia even with effective antiretroviral therapy. Microglial-neuronal interactions are of particular interest given the apparent susceptibility of dopamine neurons in the substantia nigra pars compacta (SNc) to neurodegeneration. We tested the hypothesis that the ratio of microglia to dopamine neurons in the SNc is higher than in the ventral tegmental area (VTA) and HIV-1 Tat exposure exaggerates this difference. Using the traditional biomarker tyrosine hydroxylase (TH), we examined the ratio of dopamine neurons and microglia in the VTA and SNc, the correlation between this ratio and the sensitivity of these neurons to the untoward effects of HIV-Tat. Contrary to our hypothesis, in wildtype C57BL6 mice, we found dopamine neurons in the SNc receive less microglial support than VTA dopamine neurons and that HIV-1 Tat exposure decreased TH immunoreactivity in the SNc but not in the VTA. In addition, a 7-day HIV-1 Tat induction did not induce microglial activation in the SNc or VTA; it reduced microglial density, decreased microglia-neuronal interaction and decreased TH immunoreactivity in the SNc without an effect on the total number of neurons in the SNc. Therefore the observed loss of TH immunoreactivity might be due to the loss of TH-phenotype in these neurons rather than neuronal death. Utilizing functional assays, our ongoing experiments examine the nature of neuronal-microglial interactions in the VTA and SNc, important in understanding differential susceptibility of dopamine neurons.

Disclosures: D. Miller: None. **S. Streit:** None. **K. Saha:** None. **S. Buch:** None. **W. Streit:** None. **J. McLaughlin:** None. **H. Khoshbouei:** None.

Poster

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Topic: B.05. Transporters

Support: CIHR doctoral research award

Ontario Graduate Scholarship

ERA/MRI Ontario grant

Title: Pharmacological chaperones of the dopamine transporter rescue dopamine transporter deficiency syndrome mutations in heterologous cells

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Abstract: A number of pathological conditions have been linked to mutations in the dopamine transporter gene, including hereditary Dopamine Transporter Deficiency Syndrome (DTDS). DTDS is a rare condition that is caused by autosomal recessive loss- of-function mutations in the dopamine transporter (DAT) that often affect transporter trafficking and folding. We sought to identify pharmacological chaperones of the DAT as a potential treatment for DTDS. After screening a set of known DAT ligands for their ability to increase DAT surface expression, we found that bupropion and ibogaine increased DAT surface expression, while others, including cocaine and methylphenidate, had no effect. Ibogaine and bupropion increased wild type DAT protein levels and also promoted maturation of a well-characterized ER-retained mutant K590A, suggesting an ER- level chaperoning effect. This was corroborated by pharmacologically blocking ER-exit, which eliminated the effect of ibogaine and bupropion. The rescue of K590A DAT maturation by ibogaine and bupropion is dependent on the ER-exit protein SEC24D, as knocking down or overexpressing SEC24D diminishes or enhances rescue respectively. Together the data suggest that ibogaine and bupropion stabilize a DAT conformation that promotes maturation through enhancing interaction with ER-exit machinery.Importantly, both drugs rescue DAT maturation and functional activity of the DTDS mutations A314V and R445C. Our results show that pharmacological chaperone approaches could be a viable clinical direction for increasing DAT function, particularly in DTDS and other clinical conditions associated with DAT mutations that reduce maturation. To demonstrate that these results are translational, we are currently working on a CRISPR knock-in mouse model of DTDS.

Disclosures: P. Beerepoot: None. **V.M. Lam:** None. **A. Salahpour:** None.

Poster

399. Dopamine Transporter Regulation

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Title: Role of the dopamine transporter and G protein βγ interaction in amphetamine-induced hyperlocomotion and reward-conditioned behavior in rats

Authors: *C. M. EDWARDS[1,2,3], J. C. MAUNA[1], C. D. BASSI[1], R. LUDER[1], J. A. PINO[4], J. GARCIA-OLIVARES[5], S. G. AMARA[6], G. E. TORRES[4], E. THIELS[1,2,3];
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Abstract: Dopamine (DA) plays a key role in a variety of behaviors, and aberrant levels of DA are thought to contribute to neuropsychiatric disorders, including Parkinson's disease, ADHD, and addiction. DA levels are regulated via reuptake into the presynaptic terminal by the DA transporter (DAT). Addictive psychostimulants target DAT and increase the levels of synaptic DA. Specifically, amphetamine (AMPH) reverses the direction of DA flux through DAT and causes DA efflux into the synapse. We discovered that G protein βγ subunits (Gβγ) interact with the intracellular carboxy-terminus of DAT, and that *in vitro* activation of Gβγ promotes reverse transport of DA through DAT similar to AMPH. Further, we demonstrated that pharmacological inactivation of Gβγ attenuates and pharmacological activation of Gβγ potentiates AMPH-induced hyperlocomotion. Here, we sought to extend these findings by examining: (1) AMPH-induced locomotor activity after intra-accumbal infusion of a peptide that targets a Gβγ-binding site on DAT (TAT-DATC), (2) the effect of pharmacological inactivation of Gβγ on AMPH-induced place preference, and (3) the effect of pharmacological inactivation of Gβγ on the AMPH-induced increase in DAT-Gβγ interaction. To address (1), adult male rats received intra-accumbal infusions of TAT-DATC (800ng/side) 30min before AMPH (3mg/kg, ip), and locomotor activity was measured in an open field test. To address (2), rats were trained on a standard conditioned place preference paradigm. Thirty min before AMPH (1.5mg/kg, ip) trials, rats were injected (4mg/kg, ip) or infused intra-accumbally (100ng/side) with gallein. Controls received vehicle injections/infusions prior to AMPH treatment. To address (3), rats received either gallein (4mg/kg, ip) or vehicle 30min before AMPH injections (3mg/kg, ip), and striatal tissue was harvested 30min later. DAT was immunoprecipitated and samples were probed for Gβγ. We found that: (1) blocking the interaction between Gβγ and DAT attenuated the AMPH-induced hyperlocomotion; (2) gallein pretreatment abolished the increase in preference for the AMPH-paired chamber; and (3) gallein pretreatment appears to blunt the AMPH-induced increase in Gβγ immunoreactivity. Collectively, these results suggest that the interaction between DAT and Gβγ is affected by AMPH, and that this interaction plays a critical role in AMPH-induced hyperlocomotion as well as AMPH-supported preference learning. Thus, the interaction between DAT and Gβγ presents a potential target for modulating AMPH action *in vivo*.

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Poster

399. Dopamine Transporter Regulation

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Topic: B.05. Transporters

Support: NIDA IRP

Title: Psychoactive "benzofury" compounds, 5-APB and 6-APB, mimic the effects of 3,4-methylenedioxyamphetamine (MDA) on monoamine transmission in rats

Authors: *M. H. BAUMANN[1], H. M. WALTERS[1], J. S. PARTILLA[1], B. E. BLOUGH[2], S. D. BRANDT[3];
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Abstract: The widespread non-medical use of new psychoactive substances (NPS) is a growing public health concern. In this regard, the so-called "benzofury" compounds, 5-(2-aminopropyl)benzofuran (5-APB) and 6-(2-aminopropyl)benzofuran (6-APB), are two drugs with reported stimulant-like properties in human users. Here, we compared the pharmacology of the benzofuran compounds with the structurally-related club drug, 3,4-methylenedioxyamphetamine (MDA), using *in vitro* monoamine transporter assays in rat brain synaptosomes and *in vivo* microdialysis in the nucleus accumbens of conscious male rats. We found that 5-APB and 6-APB are substrate-type releasers at dopamine transporters (DAT) and serotonin transporters (SERT), similar to the profile of effects produced by MDA. However, the benzofuran compounds are at least 3-fold more potent than MDA at evoking transporter-mediated release via DAT and SERT. When tested *in vivo*, 5-APB (0.3 and 1.0 mg/kg, i.v.), 6-APB (0.3 and 1.0 mg/kg, i.v.) and MDA (1.0 and 3.0 mg/kg, i.v.) caused dose-related elevations in extracellular dopamine and serotonin in the brain that reached 5-fold and 10-fold above baseline, respectively. The benzofuran compounds also induced profound behavioral activation characterized by forward locomotion and stereotypic movements, effects which lasted for at least 2 h post-injection. Collectively, our findings indicate that 5-APB and 6-APB are substrate-type releasers at DAT and SERT, which produce neurochemical effects similar to those produced by the club drug MDA. Importantly, the benzofuran compounds are more potent than MDA *in vitro* and *in vivo*, and produce sustained stimulant-like effects in rats. Our data suggest that benzofuran-type compounds may have significant abuse liability, and could pose risks for adverse effects, especially if used in conjunction with abused drugs or medications which enhance overall monoamine transmission in the brain.

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Poster

400. Visualizing Presynaptic Structure and Function

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Program#/Poster#: 400.01/F28

Topic: B.06. Neurotransmitter Release

Title: Structural and synaptic organization of the adult reeler mouse somatosensory neocortex

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Abstract: The *reeler* mouse with its severe structural alterations has been a useful tool to study various aspects of cortico- and synaptogenesis. This mouse has also been widely used as a model for neurological and neurodegenerative disorders such as Alzheimer's disease and lisencephaly in humans. Though, rather little is known about possible neural structural changes in the adult *reeler* mouse neocortex. For this purpose we investigated the *reeler* somatosensory cortex with a focus on the so-called 'barrel field' using serial ultrathin sectioning, high-end electron microscopy and subsequent computer-assisted 3D-volume reconstructions of synapses and their target structures. We focused on structural correlates for synaptic transmission and plasticity, in particular the shape and size of active zones and the organization and size of the pools of synaptic vesicles.
Previous studies using layer-specific cDNA probes have demonstrated that the neocortex in *reeler* lacks the typical six-layered architecture. As revealed by cytochrome C oxidase reaction, an altered 'barrel field' still exists but is organized in patches and slabs throughout the 'cortical wall'. The cortical wall is defined as the area between layer 1 (L1) and the white matter. At the light- and electron microscopic level L1 in *reeler* is dramatically reduced in volume when compared to wild type. In general, L1 is composed of clusters of 2-4 neurons often connected via gap-junctions, 'active' oligodendrocytes and astrocytes. Strikingly, the thalamocortical projection reaches the pial surface where massive fiber bundles turn and descend and then terminate throughout the entire 'cortical wall'. These fiber bundles are always accompanied by several oligodendrocytes and clusters of neurons.
The synaptic organization in *reeler* follows the same principles of a target-specific formation of synapses as described in the normal neocortex. Synaptic boutons are established on somata, dendritic shafts, spines and axon initial segments on neurons of different identities and locations within the cortical wall.
Neurons within the cortical wall form either homologous or heterologous clusters that are also connected via gap-junctions. Thus, it may be speculated that neurons of the same phenotype

determined by their genetic fate and molecular identity form these clusters as an organization principle causally related to the lack of their prospective layers. In conclusion, neurons and their synapses seems to be of greater impact for a correct wiring and the establishment of individual networks than the formation of layers.

Disclosures: **M. Prume:** None. **A. Rollenhagen:** None. **J. Lübke:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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Program#/Poster#: 400.02/F29

Topic: B.06. Neurotransmitter Release

Title: Synaptic organization in layer 5 of the human temporal lobe: A quantitative electron microscopic analysis

Authors: *R. YAKOUBI[1], A. ROLLENHAGEN[2], M. VON LEHE[3], K. SÄTZLER[4], J. LÜBKE[2,5,6];
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Abstract: Synapses are the key elements for signal transduction and plasticity in the brain, thus controlling the induction, maintenance and termination of signal transduction in any given neuronal microcircuit.
Despite a relatively large number of publications on structural and functional aspects of various synapses in the central nervous system of different animal species, very little is known about these structures in humans, in particular about their quantitative geometry. Hence, synapses in cortical layer 5 - the main output station of the neocortex and a recipient layer of thalamocortical afferents of the human temporal lobe - were investigated using serial ultrathin sectioning and digital electron microscopic images. This was followed by three dimensional (3D) volume reconstructions leading in the generation of quantitative 3D-models of synapses. We focused on structural parameters that are the most critical factors underlying synaptic transmission and plasticity, such as the shape, size, number, and distribution of active zones (AZs, functional transmitter release sites) as well as the organization and size of the three pools of synaptic vesicles, namely the readily releasable, the recycling and reserve pool. In addition, immunohistochemistry against glutamine synthetase was carried out to investigate the structural

relationship of synapses and astrocytes and thus their contribution to synaptic transmission and plasticity.
A total of 152 synaptic boutons and their target structures were completely analyzed. The majority were established either on dendritic spines (~76%) the remainder on shafts. Synaptic boutons were highly variable in both shape and size (6.20±0.77 μm^2; 0.42±0.07 μm^3**,** ranging from 0.46 to 27.33 μm^2; 0.10 to 1.93 μm^3) with a skew to middle-sized boutons. Several mitochondria (0-26) were found in the presynaptic bouton constituting ~6% of the total volume. The majority of boutons (~88%) had a single pre- (0.452±0.358 μm^2; 0.003±0,001 μm^3) and postsynaptic densities (0.405±0.100 μm^2; 0.01±0.01 μm^3), sometimes perforated. The mean total pool size of synaptic vesicles was 1580.19±255.19 (ranging from 142 to 8413) with a mean diameter of 31.99±0.87 nm. Strikingly, no correlation was found between the size of the boutons with that of mitochondria, AZs and the pool of vesicles. Synaptic complexes were surrounded by a dense network of fine astrocytic processes reaching the synaptic cleft, thus regulating the temporal and spatial glutamate concentration.
The quantitative 3D-models of synapses will lead to an improved understanding of the function of synapses in cortical networks in humans.

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Poster

400. Visualizing Presynaptic Structure and Function

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Topic: B.06. Neurotransmitter Release

Support: NINDS intramural funds

Title: Immunogold labeling of presynaptic proteins in developing hippocampal neurons

Authors: *J.-H. TAO-CHENG;
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Abstract: The transport of presynaptic proteins from soma through the axon to their final destination of synaptic terminals is complex. The present study uses preembedding immunogold electron microscopy to illustrate the trafficking of various presynaptic proteins in developing rat hippocampal neurons 3-6 days in culture. Label for synaptic vesicle (SV) integral membrane proteins, including synaptophysin, SV2, synaptotagamin and VAMP, was concentrated in the Golgi complex, and typically was associated with membranous structures in neuronal somas.

These SV proteins were then transported though axon in aggregates of pleomorphic tubular-vesicular structures, often associated with microtubules. The transport aggregates (as large as 1 μm) as well as individual vesicles/vacuoles (30-300 nm) within these aggregates were heterogeneous in size and shape. These transport aggregates are structurally different from SV clusters of uniform-sized (~40 nm) vesicles. SV clusters are formed through specialized endosomal sorting after undergoing exo- and endocytosis. SV clusters are typically located in presynaptic terminals but can also exist in axons unopposed by dendrites in young cultures. Thus, SVs with a full complement of their specific proteins are not formed in the soma but in the axon. In contrast to SV proteins, label for SV-associated proteins including synapsin I and synuclein was not concentrated at the Golgi complex, suggesting that their biosynthesis may not involve passing through it. Instead, label for synapsin I and synuclein was mostly cytosolic in neuronal somas. In axons, these labels could be cytosolic or membrane associated, including structures resembling transport aggregates for SV proteins as well as SV clusters. However, labeling intensity was preferentially higher at SV clusters, and it is known that association of synapsin I with SVs is regulated by phosphorylation. These observations suggest that the two groups of proteins, SV integral membrane proteins and SV-associated proteins, proceed through different routes of biosynthesis and axon transport, and are only colocalized in SV clusters at a later stage of SV formation.

Disclosures: J. Tao-Cheng: None.

Poster

400. Visualizing Presynaptic Structure and Function

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Challenging Exploratory Research 25670437

Title: Super resolution microscopy analysis of neuromuscular junction active zones in adult and aged mice

Authors: *Y. BADAWI[1], S. MORI[2], K. SHIGEMOTO[2], H. NISHIMUNE[1];
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Abstract: Presynaptic active zones play a pivotal role as synaptic vesicle release sites for synaptic transmission, but the molecular architecture of active zones in mammalian neuromuscular junctions (NMJs) at sub-diffraction limited resolution and the effect of aging on mammalian NMJs remains unknown. Bassoon and Piccolo are active zone specific cytosolic proteins essential for active zone assembly in NMJs, ribbon synapses, and brain synapses. Adult mammalian NMJs depend on P/Q-type voltage-gated calcium channels (VGCCs) to trigger the synaptic vesicle release. The objectives of this study are: (1) to analyze NMJ active zones using stimulated emission depletion (STED) nanoscopy and (2) elucidate how active zone proteins are altered in NMJs of aged mice. NMJ active zones in adult (8-months old) and aged (29-months old) wild-type mice were analyzed by immunohistochemical detection of active zone-specific proteins Bassoon and Piccolo, and P/Q-type VGCCs. Dual-color STED microscopy was used to image these proteins at a resolution below the diffraction limit of light microscopy.
Bassoon, Piccolo, and P/Q-type VGCCs showed punctate distribution patterns in NMJs of adult mice. Bassoon and Piccolo are thought to colocalize and share some functions at active zones, however we demonstrate an unexpected finding of non-overlapping localization of these two proteins. P/Q-type VGCC puncta colocalized with Bassoon puncta at most active zones. Interestingly, the quantity of these proteins decreased in the NMJs of aged mice. This study revealed the molecular architecture of active zones in mouse NMJs at nanoscale resolution, and shows the selective degeneration mechanism of active zone proteins in NMJs from aged mice.

Disclosures: **Y. Badawi:** None. **S. Mori:** None. **K. Shigemoto:** None. **H. Nishimune:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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Topic: B.06. Neurotransmitter Release

Support: NS090644

Title: Structural heterogeneity of presynaptic active zones underlies variability in synaptic latency across synapses at the frog neuromuscular junction

Authors: *A. E. HOMAN[1], R. LAGHAEI[3], M. DITTRICH[4], S. D. MERINEY[2];
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Abstract: The structure of presynaptic active zones (AZs), in particular the distance of voltage gated calcium channels (VGCCs) to release sensors on synaptic vesicles, can play a large role in the function of the synapse as a whole. Due to the high order relationship between external calcium concentration and the magnitude of neurotransmitter release at the neuromuscular junction (NMJ), slight variations in the organization and/or density of VGCCs at presynaptic AZs may lead to significant variability in transmitter release across synapses. We found that synaptic latency, the time between the arrival of a presynaptic action potential to the generation of a postsynaptic response, differs considerably from synapse to synapse at the frog NMJ and hypothesized that this heterogeneity is underpinned by differences in the organization/density of VGCCs at presynaptic AZs within those synapses. Using a combination of computational modeling and physiology, we manipulated the organization and/or density of VGCCs at presynaptic AZs and analyzed the effect on synaptic latency. We found that greater distances between VGCCs and release sensors in our model led to a slowing of synaptic latency while a greater density of channels resulted in faster synaptic latencies. When we blocked channels in either the model or in experiments, synaptic latency slowed. In an effort to understand these results at the AZ level, we analyzed the spatio-temporal calcium dynamics within an AZ in our MCell model during each of the manipulations. We found that when channels were moved farther from vesicle sensors, released vesicles relied more heavily on distant channels in the AZ, likely contributing to the slowing we observe in synaptic latency. When more VGCCs were added, however, nearby channels contributed most of the calcium ions and the faster synaptic latencies were likely due to an increase in calcium sources close to the released vesicle. When VGCCs were removed from our AZ model, we found that released vesicles relied primarily on the most closely associated channel and that the slowing in synaptic latency was likely due to fewer calcium sources close to the vesicle. Our combined experimental and computational data provide evidence that small changes to the organization and/or density of VGCCs can have a large influence on synaptic latency by manipulating the spatiotemporal dynamics of calcium within AZs across synapses at the frog NMJ. These data provide evidence that the structure of AZs alone can strongly influence the dynamics of neurotransmission at a synapse.

Disclosures: **A.E. Homan:** None. **R. Laghaei:** None. **M. Dittrich:** None. **S.D. Meriney:** None.

Poster

400. Visualizing Presynaptic Structure and Function

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 400.06/F33

Topic: B.06. Neurotransmitter Release

Support: CIHR MOP 86599

CIHR MOP 133602

CRC (Tier 1) to EFS

Title: Electron microscopy analysis of synaptic vesicle tethering by calcium channels at presynaptic active zones

Authors: *R. H.-C. CHEN, E. F. STANLEY;
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Abstract: Neurotransmitter is released from presynaptic terminals by calcium-gated fusion and discharge of synaptic vesicles (SVs) at active zones (AZ). Based on single-channel gated fusion we predicted that SVs are tethered to calcium channels (Stanley 1993). Using a direct, in-vitro binding assay we recently reported that SVs can bind to a 49 amino acid region towards the tip of the CaV2.2 C-terminal (Wong et al. 2013, 2014). In order to investigate tethers in situ, we developed an innovative electron microscopy (EM) technique whereby non-tethered structures were flushed out of isolated presynaptic terminals by osmotic rupture prior to imaging so that the remaining tethers and tethered SVs could be easily identified within these "synaptosomal ghosts" (Wong et al. 2014). We observed two classes of tethers that were related to the distance of the SV from the AZ: multiple-short (<45 nm) or single-long (45-175 nm) tethers. We proposed a model where the SVs are 'grabbed' from the peri-AZ cytoplasm by a long G-tether and are then 'locked' by shorter L-tethers in preparation for exocytosis. Based on its amino acid backbone, we estimate that the CaV2.2 C-terminal tail could extend to a maximum of ~210nm, making it a G-tether candidate. To test this hypothesis, we generated antibodies against the C-terminal tail and used them to immunogold label ghosts. When viewed under EM, these antibodies preferentially labeled SVs and/or their tethers. The observed pattern of labeling supports the hypothesis that the CaV C-terminal tail extend from the AZ to tether SVs.

Disclosures: R.H. Chen: None. **E.F. Stanley:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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Topic: B.06. Neurotransmitter Release

Title: Oriented docking of dense core vesicles at active zones on the presynaptic membrane of neuromuscular junctions

Authors: *J. JUNG[1], J. SZULE[2], K. STOUDER[2], U. MCMAHAN[2];
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Abstract: Electron tomography on tissue sections from fixed and stained frog neuromuscular junctions has provided evidence that active zone material, an organelle attached to the presynaptic membrane at synapses, plays a central role in the docking of synaptic vesicles on the presynaptic membrane prior to their fusing with it and releasing their neurotransmitters into the synaptic cleft. In particular, it has shown that active zone material macromolecules connect to specific sites on the membrane of undocked synaptic vesicles, stably orienting a predetermined fusion domain of the vesicle membrane toward the presynaptic membrane while bringing the two membranes together [Szule et al. (2012) PLoS ONE 7(3): e33333; Harlow et al. (2013) PLoS ONE 8(7):e69410]. As at other synapses, axon terminals at frog neuromuscular junctions contain, in addition to synaptic vesicles, vesicles that are larger, much less frequent and have a distinctive electron dense core. Dense core vesicles at neuromuscular junctions are likely to contain peptides that are released into the synaptic cleft to regulate formation and maintenance of synaptic apparatus in the muscle fibers. We show by electron tomography on adult frog neuromuscular junctions fixed at rest and during repetitive evoked synaptic activity that dense core vesicles dock on and fuse with the presynaptic membrane alongside synaptic vesicles and that active zone material connects to undocked and docked dense core vesicles in the same way it connects to synaptic vesicles. We conclude that undocked dense core vesicles, as do undocked synaptic vesicles, have a predetermined fusion domain, and that active zone material regulates the oriented docking of these structurally and functionally different vesicle types by the same macromolecular interactions. These findings are of interest not only because they concern the generality of active zone material function, but also because they may be useful for solving the related problems of whether the orientation of a predetermined fusion domain in a vesicle's membrane for contact with the presynaptic membrane provides advantages for normal vesicle membrane-presynaptic membrane fusion, the effective release of vesicle content into the synaptic cleft and/or the efficient retrieval of vesicle membrane from the presynaptic membrane for recycling.

Disclosures: J. Jung: None. **J. Szule:** None. **K. Stouder:** None. **U. McMahan:** None.

Poster

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Topic: B.06. Neurotransmitter Release

Support: CIHR MOP 86599

CIHR MOP 133602

CRC to EFS (Tier 1)

Title: Characterization of a synaptic vesicle binding site on the CaV channel distal C-terminal

Authors: *S. GARDEZI, Q. LI, A. R. NATH, E. F. STANLEY;
Genes and Develop., Krembil Res. Inst., Toronto, ON, Canada

Abstract: At fast transmitting synapses neurotransmitter is released from synaptic vesicles (SVs) that are gated to fuse with the presynaptic membrane by calcium ions that enter through voltage-gated calcium channels (CaVs). There is a growing consensus that SVs associate closely to the CaVs (Stanley Neuron 1993, TINS 2016) but the molecular linking mechanisms remain poorly understood. We developed an in vitro synaptic vesicle binding assay method (SV-PD) and showed that SVs can bind to the intact CaV2.2 channel and demonstrated pull down using the distal third, C3 segment, of its long C-terminal (Wong et al 2013). The binding site was further localized to a 49 amino acid region just proximal to the C-terminal tip (Wong et al 2014). In this study we analyzed the amino acid binding motif. The SV binding site was further restricted to a 10 amino acid sequence, HQARRVPNGY, using blocking peptides and this sequence inhibited SV recycling in chick brain nerve terminals (synaptosomes). We have identified the SV binding motif within HQARRVPNGY by means of a palette of mutant blocking peptides. While we have not as yet identified the binding target on the SV itself nor do we know the specific role of the attachment site in synaptic transmission, we hypothesize that the distal C-terminal participates in the capture of the SVs from the cytoplasm for delivery to the active zone. Further, we hypothesize that additional and later interactions secure the vesicle within range of the CaV single Ca2+ domains to prime the SV for gated fusion.

Disclosures: **S. Gardezi:** None. **Q. Li:** None. **A.R. Nath:** None. **E.F. Stanley:** None.

Poster

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Topic: B.06. Neurotransmitter Release

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Title: RIM-binding proteins are crucial for clustering calcium channels and synaptic transmission in ribbon synapse

Authors: *F. LUO[1], C. ACUNA[2], T. SÜDHOF[3];
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Abstract: Ribbon synapse transmits wide-spectrum sensory information in the visual and auditory system. Here, we assess the role of a key active zone molecule, RIM binding protein (RBP), in calcium channel clustering and neurotransmitter release using a combination of genetic, electrophysiological and ultrastructural analysis of ribbon synapse between rod bipolar cell and AII amacrine cell in the mouse retina. We find that deletion of RBP1 and RBP2 does not impact synapse fine structure, but significantly impairs presynaptic calcium influx and desynchronizes transmitter release evoked by step depolarization. We propose that RBPs play essential role in speeding and synchronizing neurotransmitter release at ribbon synapse by facilitating the clustering of calcium channels within active zones.

Disclosures: F. Luo: None. **C. Acuna:** None. **T. Südhof:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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Topic: B.06. Neurotransmitter Release

Support: NINDS-NIH U54NS083924-01

Title: Analysis of vamp7 function at the *Drosophila* neuromuscular junction

Authors: *I. D. SANTIAGO[1], B. MELENDEZ[2], T. J. LITTLETON[3], R. JORQUERA[2,4];
[1]Neurosci., Univ. Central Del Caribe, Bayamon, PR; [2]Neurosci., Univ. Central del Caribe, Bayamon, PR; [3]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [4]Inst. de Ciencias Biomedicas, Univ. de Chile, Santiago, Chile

Abstract: Vesicle-Associated Membrane Proteins (VAMPs) are SNAREs required for intracellular membrane trafficking, targeting and fusion. Tetanus insensitive (Ti)-VAMP or VAMP7 has been found in all cell types and is thought to be involved in late endosome and lysosome fusion. In neurons, VAMP7 regulates neurite outgrowth and has been implicated in

synaptic vesicle (SV) trafficking and spontaneous SV fusion. In addition, VAMP7 gene polymorphisms have been linked in patients with bipolar disorder. However, neuronal VAMP7 function and its role in neurological disorder is not clear. Combining available genetics tools, fluorescent imaging and electrophysiology we investigate VAMP7 localization and function at the *Drosophila* neuromuscular junction (NMJ). Null animals ($VAMP7^{-/-}$) generated by imprecise p-element excision are adult lethal, but survive until third instar larva displaying sluggish phenotype. More presynaptic boutons are observed in $VAMP7^{-/-}$. Strikingly, $VAMP7^{-/-}$ did not increase the average spontaneous EPSCs frequency as we expected. Nerve stimulation in $VAMP7^{-/-}$ evoked larger quantal content and synaptic depression than control. Consistently, presynaptic loadings with FM 1-43 dye revealed increased exo/endo-cycling pool and altered reserve pool formation in $VAMP7^{-/-}$. Directed expression of VAMP7-GFP in motor neurons or postsynaptic muscles localizes the fusion protein at the plasma membrane and in intracellular compartments around the nuclei. Localization analysis at the NMJ reveal presynaptic puncta and enrichment at the sub-synaptic area. Our findings indicate altered presynaptic function in $VAMP7^{-/-}$ and a putative role in retrograde signaling at *Drosophila* NMJ.

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Poster

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Support: 4 R37 NS036251-18

5 F32 NS090727-02

Title: Dynamics of the Extended Synaptotagmins in the endoplasmic reticulum of neurons

Authors: *C. WHITEUS[1], J. WANG[2], R. YASUDA[2], P. DE CAMILLI[3];
[1]Yale Univ., New Haven, CT; [2]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [3]Depts. Neurosci. and Cell Biology, Kavli Inst. for Neurosci., Yale University, Howard Hughes Med. Inst., New Haven, CT

Abstract: The endoplasmic reticulum (ER) is the site of synthesis of most bilayer lipids. From the ER, newly synthesized membrane lipids are delivered to the plasma membrane via vesicular transport in the secretory pathway. Conversely, insoluble lipid metabolites can return to the ER

for metabolic recycling via the endocytic pathway. This bidirectional traffic requires transit through the Golgi complex and involves at least minutes, but can be much longer in the case of distal neuronal compartments. A direct and more rapid exchange of lipids between the ER and the plasma membrane via lipid transport proteins and independent of membrane traffic also occur. Growing evidence shows that much of this transport occurs via close appositions between the ER and the plasma membrane mediated by protein tethers that contain lipid "harboring" modules. The Extended Synaptotagmins (E-Syts) are a set of three ubiquitously expressed intrinsic ER proteins that function as such tethers. They bind the plasma membrane via their C2 domains, bridging the two membranes in a Ca^{2+} and $PI(4,5)P_2$ regulated manner. Additionally, they have a lipid transport function mediated by their SMP domain. Here we show that GFP-tagged E-Syt constructs localize to soma, axons, dendrites, and dendritic spines of neurons. GFP-E-Syts 2, 3, and fluorescently tagged E-Syt 2/3 heterodimers are constitutively present at ER-plasma membrane contact sites in neurons, however tagged E-Syt1 and E-Syt1/E-Syt2 heterodimers are recruited to these contacts when neurons are stimulated chemically (with glutamate or NMDA) or electrically. Using a FRET-based method, we observed transient GFP-E-Syt recruitment to the plasma membrane in response to local glutamate uncaging. Glutamate uncaging also induced transient migration of the ER into spines that previously did not contain ER. The rapid recruitment of ER-anchored E-Syts following stimulation-induced Ca2+ entry suggests that neuronal activity and plasticity is accompanied by transfer of lipids between the ER and the plasma membrane.

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Poster

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GSLS Wuerzburg Grant

Title: Genetic expression of an active zone peptide to induce cell-specific synaptic depression and to screen for vesicle tethering factors

Authors: *R. J. KITTEL[1], N. SCHOLZ[1], N. EHMANN[1], C. STIGLOHER[2], T. LANGENHAN[1];
[1]Inst. of Physiology, Dept. of Neurophysiol., [2]Div. of Electron Microscopy, Biocenter, Univ. of Wuerzburg, Wuerzburg, Germany

Abstract: Bruchpilot (Brp) is a core protein component of the *Drosophila* active zone (AZ) where it promotes calcium channel clustering to ensure adequate transmitter release probability (Kittel et al., 2006). In addition, the very C-terminal region of Brp supports vesicle tethering to the AZ cytomatrix. In a C-terminally truncated allele, brp^{nude} (lacking the last 17 amino acids), impaired vesicle tethering is accompanied by short-term synaptic depression, impaired sustained transmitter release, and a slowed recovery phase (Hallermann et al., 2010).
We set out to test the hypothesis that neuronal expression of a peptide containing the Brp C-term would deliver a synaptic phenocopy of brp^{nude} by competitively binding the putative vesicular interaction partner(s) of Brp. Our electrophysiological analysis of larval neuromuscular synapses supports this hypothesis and sets the basis for a subsequent *in vivo* screen to identify the interacting protein(s). To this end, a membrane-bound Brp C-term was neuronally expressed to enrich synaptic vesicles at ectopic locations. Different RNAi lines against vesicle-associated proteins were then screened and scored for their ability to revert the ectopic vesicle localisation.
References
Hallermann S, Kittel RJ, Wichmann C, Weyhersmüller A, Fouquet W, Mertel S, Owald D, Eimer S, Depner H, Schwärzel M, Sigrist SJ, Heckmann M (2010) Naked dense bodies provoke depression. J Neurosci 30:14340-14345.
Kittel RJ, Wichmann C, Rasse TM, Fouquet W, Schmidt M, Schmid A, Wagh DA, Pawlu C, Kellner RR, Willig KI, Hell SW, Buchner E, Heckmann M, Sigrist SJ (2006) Bruchpilot promotes active zone assembly, Ca^{2+} channel clustering, and vesicle release. Science 312:1051-1054.

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Poster

400. Visualizing Presynaptic Structure and Function

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Support: 31190061

Title: Proteomic screening of GABAergic and glutamatergic neurons isolated by fluorescence activated sorting

Authors: *Z. WEI[1], X. LI[1,2], L. QIN[1], H. YU[1], Z. GAO[1], S. DUAN[1];
[1]Inst. of Neuroscience, Zhejiang Univ., Zhejiang, China; [2]Nantong Univ., Nantong, China

Abstract: Abstract: We established a novel Fluorescence Activated Cell Sorting (FACS) method to purify two major types of neurons in the brain, GABAergic and Glutamatergic neurons. The cleavage of neurons from GAD67-GFP (+/-) on DIV 14-16 were used to investigate the profile of purified GABAergic and glutamatergic neurons samples by Label-free LC-MS/MS. We identified 204 enriched proteins in FACS samples, of which SV2B mainly distributes in glutamatergic Synapse, while SV2C and VAMP1 mainly distribute in GABAergic synapse. Thus, our work indicates that only relatively fewer synaptic proteins are differentially distributed in glutamatergic and GABAergic neurons, which may play important roles in determining differential properties in two types of neurons.

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Poster

400. Visualizing Presynaptic Structure and Function

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Title: Synaptotagmin1 sorting to synaptic vesicles is probabilistic

Authors: *T. A. SCHIKORSKI[1], D. CRUZ[2];
[1]Univ. Central Del Caribe, Bayamon, PR; [2]Univ. Puerto Rico, San Juan, PR

Abstract: Protein sorting is the very foundation for the genesis of functional distinct cell organelles. At synapses, protein sorting is crucial for the genesis of 'perfect' synaptic vesicles (SV's) that ensure activity-dependent release of neurotransmitter. Many molecular pathways

have been identified that support regulated SV protein sorting but despite these advances, a quantifiable mechanistic model for protein sorting is still missing. In previous studies protein sorting was investigated by exploiting molecular methods followed by high resolution fluorescence imaging. Here, we analysed SV protein sorting on the ultrastructural level by exploiting a new genetically encoded label for electron microscopy with unprecedented high sensitivity, enhanced horseradish peroxidase (eHRP). eHRP is a HRP variant with elevated catalytic activity that is reliably detected in mammalian neurons.
Synaptotagmin 1 is key to activity-dependent SV fusion and failure to sort synaptotagmin 1 will render SV's disfunctional. We tagged synaptotagmin 1 with eHRP and expressed eHRP-synaptotagmin 1 in cultured hippocampal neurons. eHRP-synaptotagmin1 abundantly labeled SVs in many neurons which allowed us to analyse its distribution. eHRP positive SV's spread with no preference in the presynaptic bouton and mixed randomly with the main SV cluster. Also, we did not detect any preferential sorting of eHRP-synaptotagmin1 to one of the functionally defined SV pools and labeled SV's had the same chance as unlabeled SV's to dock at active zones. This data suggested a random distribution of synaptotagmin 1 and not heavily regulated sorting.
To determine the underlying mechanism for synaptotagmin 1's probabilistic distribution we switched to pre-embedding immunogold and measured the synaptotagmin 1 copy number for each SV's at individual presynaptic boutons in cell cultured neurons and *in situ*. Frequency histograms of the copy number per SV were best fitted with a Poisson distribution which is the result of a single probabilistic mechanism that distributes X protein copies to Y SV's. Because the mathematical basis for this process is well known this mechanism can serve as a quantifiable mechanistic model for SV protein 'sorting'. Furthermore, the model can explain molecular distinct SV pools and is useful to estimate protein translation rates in neurons.

Disclosures: **T.A. Schikorski:** None. **D. Cruz:** None.

Poster

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Title: Distinct Ca^{2+} dynamics in glutamatergic and aminergic synapses determined by intrinsic neuronal properties independent of synaptic bouton physical dimensions and GCaMP expression levels.

Authors: *X. XING[1], C.-F. WU[2];
[1]Dept. of Biol., Univ. of Iowa, Iowa City, IA; [2]Dept. of Biol., the Univ. of Iowa, Iowa City, IA

Abstract: Our previous work demonstrates distinct stimulus frequency responses of Ca2+ dynamics in glutamatergic tonic type Ib, phasic type Is synapses, and octopaminergic type II synapses in the Drosophila larval neuromuscular junction (NMJ). A fundamental question is whether such distinction is due to intrinsic differences in Ca2+ handling properties of these synaptic boutons, or results from other factors. In principle, the recorded Ca2+ transients can be influenced by bouton physical dimension differences, and variations in the Ca2+ indicator sensitivity, expression level, and cellular localization. In this study, Ca2+ transient amplitude and kinetics from each bouton were individually measured and correlated with bouton size and baseline GCaMP fluorescence intensity. We examined adjacent type Ib, Is and II boutons in the same microscopic field, using cytosolic indicators of different sensitivity (GCaMP1 and 6) as well as membrane-bound GCaMP5. The results show that even though significant variation in bouton sizes exists in each type of synaptic terminals, the maximum fluorescence change (ΔF/F) and the rise and decay kinetics were uniform among boutons along the synaptic terminals, displaying distinct properties characteristic of each specific type. Furthermore, GCaMP expression level may very between synaptic terminals in different larvae, as indicated by basal fluorescence reading without nerve stimulation. However, ΔF/F and rise and decay kinetics remain characteristic of each bouton type. Our data also indicate significantly slower decay kinetics of type II boutons, suggesting a Ca2+ clearance mechanism distinct from type I boutons. Further, examination of hyperexcitability mutations, e.g. eag Sh K+ channel double mutant and bss Na+ channel mutant demonstrated characteristic effect of each mutations, regardless of bouton physical dimension and the GCaMP indicator expression levels. Hyperexcitability enhanced the rise, but not decay, kinetics of Ca2+ transients, reflecting larger Ca2+ influx. Our results show that bouton size variation and choice of the versions of GCaMP indicators do not interfere with the determination of intrinsic synaptic properties of different types of boutons, their characteristic ΔF/F, rise and decay kinetics can be reliably determined with Ca2+ transient measurements from GCaMP signals.

Disclosures: X. Xing: None. **C. Wu:** None.

Poster

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Title: Signaling pathway controlling mitochondria-dependent presynaptic calcium clearance and neurotransmitter release properties at single synapses along cortical axons

Authors: *S.-K. KWON[1], R. SANDO, III[2], T. L. LEWIS, Jr[1], Y. HIRABAYASHI[1], A. MAXIMOV[2], F. POLLEUX[1];
[1]Columbia Univ., New York, NY; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Individual synapses vary significantly in their neurotransmitter release properties and underlies complex information processing by neural circuits. Presynaptic Ca^{2+} homeostasis plays a critical role in specifying neurotransmitter release properties. Interestingly, several studies revealed that action potential (AP)-evoked presynaptic Ca^{2+} signals differ drastically between individual boutons along the same axons. However, the cellular and molecular pathways regulating Ca^{2+} dynamics in a synapse-specific way are still poorly understood. It has been suggested that mitochondria are involved in presynaptic Ca^{2+} clearance, but the impact on modulation of neurotransmitter release properties varies in different species and neuron subtypes. Also, the signaling pathways regulating presynaptic mitochondrial function in this context are largely unknown. We and others identified that the serine/threonine kinase LKB1 is a master regulator of axon morphogenesis in the mammalian central nervous system. LKB1 is necessary and sufficient for axon formation, and also LKB1 plays an essential role in terminal axon branching *in vivo* through the regulation of presynaptic mitochondrial capture. These latest results raised a central unresolved question regarding the relevance of presynaptic mitochondria in axon morphogenesis. To investigate the role of presynaptic mitochondria, we developed genetically-encoded Ca^{2+} sensors targeted to the mitochondrial matrix (mito-GCaMP5G) or to presynaptic boutons (vGlut1-GCaMP5G) of layer 2/3 cortical pyramidal neurons, and we observed that the presence or absence of mitochondria at presynaptic boutons regulate presynaptic Ca^{2+} levels. In addition, using synaptophysin-pHluorin, we revealed that neurotransmitter release properties at individual presynaptic boutons correlate the presence or absence of mitochondria. Finally, we identified that the LKB1 controls presynaptic Ca^{2+} homeostasis through regulation of the abundance of the mitochondrial calcium uniporter (MCU). Disruption of this signaling pathway leads to increased presynaptic Ca^{2+} accumulation and drastic changes in neurotransmitter release properties, including: (1) increased rate of spontaneous vesicle fusion, (2) augmentation of asynchronous mode of evoked neurotransmitter release, and (3) abrogation of short-term synaptic depression during trains of action potentials (AP). Our results provide novel insights into the cellular and molecular mechanisms whereby mitochondria control neurotransmitter release properties in a synapse-specific way through presynaptic Ca^{2+}clearance.

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Poster

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Title: Quantification of fast presynaptic Ca^{2+} kinetics using non-stationary single compartment model

Authors: Y. TIMOFEEVA[1], D. RUSAKOV[2], *K. E. VOLYNSKI[2];
[1]Dept. of Computer Sci. and Ctr. for Complexity Science, Univ. of Warwick, Coventry, United Kingdom; [2]UCL Inst. of Neurol., London, United Kingdom

Abstract: Fluorescence imaging is an important tool in examining Ca^{2+}-dependent machinery of synaptic transmission. Classically, deriving the kinetics of free Ca^{2+} from the fluorescence recorded inside small presynaptic boutons has relied on singe-compartment models of Ca^{2+} entry, buffering and removal. In many cases, steady-state approximation of Ca^{2+} binding reactions in such a model allows analytical solutions for the Ca^{2+} kinetics in question. However, the fast rate of action potential-driven Ca^{2+} influx is often comparable with the rate of Ca^{2+} buffering inside the presynaptic terminal. In this case, computations that reflect non-stationary changes in the system might be required for obtaining essential information about rapid transients of intracellular free Ca^{2+}. Based on the experimental data we propose an improved procedure to evaluate the underlying presynaptic Ca^{2+} kinetics. We show that in most cases the non-stationary single compartment model provides accurate estimates of action-potential evoked presynaptic Ca^{2+} concentration transients, similar to that obtained with the full three dimensional diffusion model. We also develop a computational tool aimed at stochastic optimisation and cross-validation of the kinetic parameters based on a single set of experimental conditions. The proposed methodology provides robust estimation of Ca^{2+} kinetics even when a priori information about endogenous Ca^{2+} buffering is limited.

Disclosures: **Y. Timofeeva:** None. **D. Rusakov:** None. **K.E. Volynski:** None.

Poster

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NSERC

Title: Presynaptic calcium dynamics translate bursts of action potential to control the mode of neurotransmitter release at the mossy fiber to CA3 pyramidal cell synapse

Authors: *S. CHAMBERLAND, A. EVSTRATOVA, K. TOTH;
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Abstract: Neurons encode information in the number and frequency of action potentials they discharge. Hippocampal granule cells fire infrequently, but discharge bursts of action potential with highly variable frequencies. At the hippocampal mossy fiber to CA3 pyramidal cell synapses, synchronous release, short-term facilitation and asynchronous release actively contribute to the neuronal dialogue. It remains undetermined how giant MF presynaptic terminals translate the number and the frequency of incoming action potentials into specific calcium dynamics to encode distinct modes of release. To investigate this question, we used a combination of electrophysiology and random-access two-photon presynaptic calcium imaging in large MF terminals in acute mouse hippocampal slices. First, we explored the spatiotemporal dynamics of calcium elevations in giant MF terminals. Bursts of 10 APs evoked at 100 Hz increased the intraterminal calcium concentration significantly more than when bursts were evoked at 20 Hz. Interestingly, spatial homogenization of calcium in presynaptic terminals during trains was identical for APs evoked at both frequencies. Next, we observed whether different modes of release could be supported by these specialized calcium dynamics. Repetitive electrical stimulation of mossy fibers (10 stimuli) at 20 Hz and 100 Hz facilitated synchronous EPSCs up to a similar maximum, but facilitation was faster and reached a plateau with 100 Hz stimulation. On the other hand, the frequency of asynchronous release was larger when EPSCs were evoked at 100 Hz than at 20 Hz. Altogether, these results suggest that facilitation of synchronous release is dependent on the number of APs in the trains and is encoded through spatial homogenization of calcium. On the other hand, asynchronous release is dependent on the number of APs and their average frequency, which is encoded in the total peak calcium.

Disclosures: S. Chamberland: None. **A. Evstratova:** None. **K. Toth:** None.

Poster

400. Visualizing Presynaptic Structure and Function

Location: Halls B-H

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Support: ZIA NS003009-13

ZIA NS003105-08

Z01-DC000002

ZIC DC000081

Title: Hemi-fused structure mediates and controls fusion and fission in live cells

Authors: *S. A. VILLARREAL[1], W.-D. ZHAO[1], E. HAMID[1], P. J. WEN[1], E. S. KRYSTOFIAK[2], H.-C. CHIANG[1], B. KACHAR[2], L.-G. WU[1];
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Abstract: Membrane fusion and fission are vital to eukaryotes' life. For three decades, it has been proposed that fusion is mediated by fusion between proximal leaflets of two fusing bilayers (hemi-fusion) that produces a hemi-fused structure, followed by fusion between distal leaflets, whereas fission is via hemi-fission, which also produces a hemi-fused structure, followed by full fission. However, owing to the lack of direct observation of hemi-fusion/hemi-fission in live cells, whether hemi-fusion/hemi-fission mediates fusion/fission in live cells is unclear. Whether the hemi-fused structure is the dead end of fusion or serves to prime vesicles for fusion remains unresolved. A competing hypothesis involving the formation of a protein-lined pore has been proposed as the fusion mechanism. With confocal and super-resolution STED microscopy, here we observed the hemi-fused Ω-shaped structure for the first time in live cells (neuroendocrine chromaffin cells). This structure could be generated from either fusion between vesicles and the plasma membrane or fission of fully fused Ω-profiles. Unexpectedly, its transition to full fusion or fission was determined by the competition between fusion and calcium/dynamin-dependent fission mechanisms, and was surprisingly slow (seconds to tens of seconds) in a significant fraction of the events. These results provide evidence missed over the last three decades, proving the hemi-fusion and hemi-fission hypothesis in live cells. Our findings establish a live-cell model of fusion and fission, in which the hemi-fused intermediate is the key structure controlling fusion/fission, as fusion and fission mechanisms compete to determine the transition of the hemi-fused intermediate to fusion or fission.

Disclosures: **S.A. Villarreal:** None. **W. Zhao:** None. **E. Hamid:** None. **P.J. Wen:** None. **E.S. Krystofiak:** None. **H. Chiang:** None. **B. Kachar:** None. **L. Wu:** None.

Poster

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Topic: B.06. Neurotransmitter Release

Support: R01 MH099557

Title: Activity-dependent movement of synapsin between *Drosophila* motor boutons

Authors: *A. VASIN, M. BYKHOVSKAIA;
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Abstract: Synapsin (Syn) is an abundant phosphoprotein that regulates synaptic vesicle clustering, synaptic plasticity, and neuronal development. Our earlier study demonstrated that Syn promotes the activity-dependent outgrowth of new boutons in the *Drosophila* neuromuscular junction (NMJ), and that this mechanism may involve Syn movement and redistribution between synaptic boutons. To investigate this mechanism directly, we generated a *Drosophila* line expressing Syn tagged with a photo-activateable mCherry marker. Employing this line, we selectively activated the mCherry tag within individual boutons and tracked synapsin movement along the NMJ for a period of one hour. We found that Syn did move between synaptic boutons, and that this movement depended on the neuronal activity. At rest, we were able to observed Syn movement towards neighboring boutons in some of the preparations. In contrast, high frequency (10 or 30 Hz for 30 min) stimulation of the nerve, as well as high K^+ depolarizations, produced Syn movement over the NMJ length of 10-15 μm. These stimulation paradigms typically included Syn redistribution over 3-5 synaptic boutons. Interestingly, the movement continued after the end of the stimulation and during the entire time period analyzed (1 hour). These results suggest a novel Syn function in the mechanisms of signaling and trafficking between synaptic boutons, which can be triggered by intense neuronal activity.

Disclosures: **A. Vasin:** None. **M. Bykhovskaia:** None.

Poster

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Support: MRT

FDT20150532260

DEQ20140329498

Title: Monitoring vesicle dynamics in gaba-depleted hippocampal neurons using vgat-phluorin.

Authors: *L. BONET, S. SUPPLISSON;
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Abstract: There is still no comprehensive understanding of the molecular organization and mechanisms that regulate the filling of GABA-containing vesicles during their continuous recycling at inhibitory terminals. In hippocampal neuronal cultures, electrophysiological recordings in pairs and FM5-95 uptakes show a progressive reduction in the pool of recycling vesicles when GABAergic neurons run out of transmitter, whereas restoring GABA supply in GABA-depleted terminals reinstates normal vesicle cycling and filling (Wang et al., Neuron 80:143, 2013).
Here, we used VGAT-pHluorin for monitoring vesicle recycling during long trains of action potentials. VGAT (also named VIAAT) is the vesicular transporter shared by all inhibitory interneurons with its C-terminus located inside the vesicle lumen. In resting condition, the fluorescence of the pH-sensitive GFP (super ecliptic pHluorin) fused to the VGAT C-terminus is quenched by the acidic vesicular pH (Santos et al., J Neurosci 33:10634, 2013).
We expressed VGAT-pHluorin in neuronal cultures derived from rat hippocampus, and performed electrophysiological recordings and imaging ≥7 days post transfection. Brief application of NH4 reveals a punctate distribution of VGAT-pHluorin in axonal varicosities with a high signal-to-noise ratio (x2 to 4) using a high-resolution sCMOS camera. Extracellular acidification with MES (2-(N-morpholino) ethanesulfonic acid) shows low background expression of VGAT-pHluorin at the plasma membrane. Trains of action potentials at 20Hz evoked by electrical stimulation of the transfected neuron activate the same varicosities as NH4 but with lower intensity, as expected if only a fraction of the vesicles undergo exocytosis.
Using low transfection rate, it is possible to identify and whole-cell patch putative pairs of synaptically connected neurons. Surprisingly, evoked post synaptic currents recorded in the presence of NBQX 2µM and MK801 5µM were inhibitory in all pairs tested, blocked by SR 10µM, suggesting that overexpression of VGAT-pHluorin might be toxic in excitatory neurons.

The large presynaptic response evoked with 300 APs at 20 Hz showed modest steady release of GABA, presumably because of the large synaptic depression at this frequency. This suggests that vesicles if recycled, could not be fully filled with GABA. In contrast, we observed a steady but lower fluorescent signal during 4Hz stimulation as expected when endocytosis compensates for exocytosis.
These results confirm that VGAT-pHluorin is a useful probe to monitor vesicle recycling in GABAergic interneurons.

Disclosures: L. Bonet: None. **S. Supplisson:** None.

Poster

400. Visualizing Presynaptic Structure and Function

Location: Halls B-H

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Program#/Poster#: 400.22/F49

Topic: B.06. Neurotransmitter Release

Title: *In vivo* time lapse imaging of axonal dense core vesicle trafficking in anaesthesized and awake mice

Authors: *J. KNABBE[1], J. NASSAL[1], H. HORSTMANN[1], M. VERHAGE[2], T. KUNER[1]; [1]Inst. of Functional Neuroanatomy, Heidelberg, Germany; [2]Dept. of Functional Genomics, Ctr. for Neurogenomics and Cognitive Res., Amsterdam, Netherlands

Abstract: Dense core vesicles (DCV) are large, electron-dense vesicles designed to transport a variety of cargo molecules, including neuropeptides or neurotrophins, from their site of production at the neuronal soma towards their respective release sites in dendrites or axons. Trafficking and movement characteristics of DCVs have been typically investigated in chromaffin cells or primary cell culture models. Yet, DCV trafficking characteristics and their responsivity to physiological release signals could differ fundamentally in the mammalian brain *in vivo* due to the densely packed tissue neuropil, neuronal connectivity, neuron-glial interactions or different functional brain states. In this study, we used multiphoton-*in*-*vivo*-imaging to visualize DCV trafficking in the central nervous system in anaesthesized or awake mice through a chronically implanted cranial window. Viral co-expression of live fluorescent DCV-markers and axonal markers in thalamic projection neurons allowed us to specifically visualize axonal projections from the thalamus to upper layers of the cortex. Ultrastructural features of the labeled vesicles were assessed using electron-microscopy and photooxidation of fluorescent DCV- and axon markers. Taking advantage of semiautomated and automated tracking approaches we were able to analyze the movement of hundreds of vesicles in individual axons of different animals. These data for the first time reveal speed, directionality and number of moving DCVs and their

movement characteristics at axonal en passant boutons *in vivo* in awake and anaesthesized mice. This approach will enable future studies of the physiological mechanisms triggering DCV cargo-release and studies examining DCV trafficking in pathophysiological situations potentially linked to defects in DCV trafficking, such as neurodegenerative disorders and the aging brain.

Disclosures: J. Knabbe: None. **J. Nassal:** None. **H. Horstmann:** None. **M. Verhage:** None. **T. Kuner:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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Topic: B.06. Neurotransmitter Release

Support: Takeda Science Foundation

Title: Dynamics of synaptic vesicle protein after single vesicle exocytosis at a hippocampal presynaptic active zone recorded by a novel live-cell imaging method

Authors: *J. FUNAHASHI, H. TANAKA, T. HIRANO;
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Abstract: In presynaptic terminals neurotransmitter is repetitively released depending on patterns of action potential firing. To support multiple rounds of release, synaptic vesicles and their proteins need to be recycled efficiently. However, molecular mechanisms to regenerate synaptic vesicles are not fully elucidated. Especially, how synaptic vesicle proteins are retrieved after exocytosis remains unclear. To address this issue, we have developed a novel live-cell imaging method and aimed to monitor dynamics of synaptic vesicle proteins after single vesicle exocytosis at a hippocampal presynaptic active zone. We used total internal reflection fluorescence microscopy (TIRFM) that allows observation of fluorescent molecules with a high signal-to-noise ratio by limiting the depth of excitation field to approximately 100 nm. To visualize fluorescent molecules at active zones with TIRFM, we induced active zone formation on the glass surface. We coated cover glass with a synaptic adhesion molecule Neuroligin (postsynaptic membrane protein that can induce presynaptic differentiation). Afterward, we cultured rat hippocampal neurons on Neuroligin-coated glass. To examine whether active zones are formed on the glass surface, we transfected EGFP and TagRFPt-CAST to neurons. TagRFPt-CAST is a fusion protein of a red fluorescent protein TagRFPt and an active zone scaffold protein CAST. Many TagRFPt-CAST positive axonal areas were observed with TIRFM, suggesting presynaptic structures were formed directly on the Neuroligin-coated glass. Then, we

transfected TagRFPt-CAST and SypHy (synaptic vesicle protein Synaptophysin tagged with a pH-sensitive fluorescent protein Super Ecliptic pHluorin) to neurons. Whereas SypHy does not show fluorescence in acidic synaptic vesicles, it becomes brighter upon exocytosis. We applied an electrical field stimulation to trigger exocytosis and recorded the SypHy signal around a CAST-labeled active zone. An increase in the SypHy signal at the active zone coincident with the stimulation, was observed in about 10% of trials. We presumed that such an event corresponded to exocytosis of single synaptic vesicles. The SypHy signal decreased within about 100 ms in most cases. The time course of SypHy signal decrease could be classified into two patterns. In one pattern the SypHy signal spread and decayed, suggesting Synaptophysin diffused out from the release site at the active zone. In another pattern the SypHy signal rapidly disappeared without signal spread, which might correspond to kiss-and-run exo-endocytosis.

Disclosures: **J. Funahashi:** None. **H. Tanaka:** None. **T. Hirano:** None.

Poster

400. Visualizing Presynaptic Structure and Function

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AHA14PRE20380168

Title: Rapid and spatially-confined PI(4,5)P_2 manipulations by optogenetic approaches regulate vesicle docking and secretion.

Authors: ***C. JI**, F. FAN, X. LOU;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Phosphoinositides in the plasma membrane (PM) regulate vesicle trafficking and secretion. As a key PM determinant phospholipid, PI(4,5)P_2 is required for both hormone secretion and transmitter release. However, the molecular and cellular mechanism in which PI(4,5)P_2 directly regulates vesicle trafficking and secretion remains poorly understood. The available approaches, such as genetic perturbations or pharmacological inhibitors, often suffer from chronic compensation or off-target and complicate the interpretations of data. Here,

utilizing the latest optogenetic tools based on the blue light-inducible molecule demonization and translocation, we developed a novel approach to rapidly and specifically manipulate $PI(4,5)P_2$ levels in the PM. We achieved rapid and efficient decrease of the $PI(4,5)P_2$ levels within several seconds in INS-1 cells, a secretory cell model that is widely used to study the regulated insulin secretion. Combing with TIRF imaging, we characterize the properties of the light-induced $PI(4,5)P_2$ level changes and their impacts on different steps of insulin granule trafficking. A global decrease of $PI(4,5)P_2$ level enhances insulin granule dynamics, inhibits the evoked increase in intracellular Ca^{2+} concentrations, and impairs secretion. To mimic the physiological condition where $PI(4,5)P_2$ often changes locally rather globally, we have designed an approach to selectively decrease the local $PI(4,5)P_2$ levels at vesicle docking sites on the PM. Preliminary data from this approach reveals a selective defect in vesicle docking, without a detectable change in global $PI(4,5)P_2$ levels. This approach provides a new step toward understanding the lipid signaling in vesicle trafficking in secretory cells; it can be adapted for the study of other phosphoinositides in cell signaling. Our data suggest that $PI(4,5)P_2$ mediated vesicle docking might be a rate-limiting step of the regulated secretion under physiological conditions.

Disclosures: **C. Ji:** None. **F. Fan:** None. **X. Lou:** None.

Poster

401. Spike-Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust

Institute of Neuroscience

Newcastle University

Title: Spike-timing dependent plasticity in the long latency stretch reflex following paired stimulation from a wearable electronic device

Authors: *K. FOYSAL, F. DE CARVALHO, S. BAKER;
Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: The reticulospinal tract contributes to control hand and arm movements in primates and plays a key role in functional recovery after corticospinal lesion. Here we tested the ability of non-invasive paired stimulation delivered by a wearable electronic device to produce plastic changes in reticulospinal output. We first recorded the long-latency stretch reflex (LLSR) from

elbow flexor muscles of healthy adult human subjects following an extensor perturbation as a putative measure of reticulospinal output. Subjects were then fitted with the portable device which delivered auditory click stimuli through an earpiece, and electrical stimuli around motor threshold to the biceps muscle via surface electrodes. Auditory clicks are known to generate action potential bursts in reticulospinal neurons, which are also activated by peripheral afferents. We tested four paradigms: biceps stimulus 10 ms before click (Bi-10ms-C); click 25 ms before biceps (C-25ms-Bi); click alone (C only); biceps alone (Bi only). Average stimulus rate was 0.67 Hz. Subjects left the laboratory wearing the device, and performed normal daytime activities. Around 7 hours later, they returned and stretch reflexes were re-measured to compare with the measurements taken before. The LLSR was significantly enhanced in the biceps muscle (on average by 49%) after the Bi-10ms-C paradigm, but suppressed for C-25ms-Bi (by 36%). For the same muscle, Bi-only and C-only did not induce any change in the LLSR. No changes occurred in the LLSR for the brachioradialis muscle while testing any of the four paradigms.
We conclude that paired stimulation with precisely adjusted order and time intervals was able to strengthen or weaken LLSR pathways selectively for the stimulated muscle. This result is consistent with spike-timing dependent plasticity and may reflect changes in reticulospinal output. This is the first demonstration that such circuits may be modifiable via paired-pulse methods, and could open up new possibilities in motor systems neuroscience and rehabilitation.

Disclosures: K. Foysal: None. **F. De Carvalho:** None. **S. Baker:** None.

Poster

401. Spike-Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Swiss National Science Foundation FN 31003A-138526

Title: The role of local excitatory networks in the lateral amygdala in emotional memory learning

Authors: *M. ABATIS[1], R. NIU[1], R. PERIN[2], H. MARKRAM[2], H. BITO[3], R. STOOP[1]; [1]Dept. of Psychiatry, CHUV, Prilly, Switzerland; [2]BMI, EPFL, Lausanne, Switzerland; [3]Dept. of Neurochemistry, Univ. of Tokyo, Tokyo, Japan

Abstract: Introduction: Fear conditioning combines an unconditioned stimulus with a conditioned stimulus (CS) so that the CS alone can subsequently elicit fear-related responses. While the convergence of signals onto single lateral amygdala (LA) neurons has been

extensively studied, little is known about the role of connections between LA neurons in threat memory encoding. Indeed, recent findings in the hippocampal CA3 highlight the role of recurrent networks in memory encoding (Rajasethupathy et al., Nature, 2015), prompting further investigation.
Aims: We hypothesized that threat-related signals are re-integrated in the LA through local neuronal assemblies. To address this, we aimed to characterize the LA's network organization, including connectivity among memory-participating neurons.
Methods: We used whole-cell patch-clamp recordings to simultaneously access up to 12 neurons at a time. The connectivity of over 563 neurons was assessed by delivering, successively, trains of 8 pulses at 20 Hz and monitoring for induced post-synaptic potentials. Neuronal memory-recruitment was assessed by expressing a destabilized GFP under an enhanced Arc promoter, after threat memory recall.
Results: We observed ~2% connectivity biased towards close-proximity neurons. Analysis of the peak excitatory post-synaptic current amplitudes by simple binomial analysis suggested 1-5 release sites, with a quantal size of 10±3 pA and probability of release of ~0.5 ± 0.2 (±SD).
To better understand how this network could encode fearful memories, we performed a spike-timing-dependent plasticity protocol which resulted in a 30% increase in amplitude for the first excitatory post-synaptic potential (EPSP) of the stimulus train, while average EPSP amplitude was unchanged, suggesting a redistribution of synaptic efficacy.
Finally, we assessed connectivity among neurons participating in the recall of a threat memory trace. Recruited neurons had greater connectivity (4%), higher EPSP amplitude (1.4±0.1 mV) and higher probability to observe an EPSP (0.5±0.04 %) compared to controls (2%, 0.9±0.1mV and 0.4±0.01%, respectively; ±s.e.m.). This suggests that either stronger and more numerous connections enhance local network excitability and favor memory recruitment or that the observed changes result from memory recruitment.
Conclusion: The LA network follows a "small-world" network organization, with memory-recruited neurons forming stronger and more numerous connections. This suggest either that enhanced local network excitability favors memory recruitment or that memory recruitment increases connectivity, to be determined by future experiments.

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Poster

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Support: R01 MH081935

Title: Postsynaptic calcium dynamics associated with bidirectional plasticity of NMDA receptor-mediated transmission

Authors: S. LUTZU[1], *K. ALVINA[2], P. CASTILLO[1];
[2]Neurosci., [1]Albert Einstein Col. of Med., Bronx, NY

Abstract: NMDA receptors (NMDARs) mediate fast excitatory transmission and are crucial for normal neural circuit development and cognitive processes such as learning and memory. While classically considered triggers of long-term plasticity (LTP/LTD) of AMPA receptors (AMPARs), NMDARs themselves can be dynamically regulated by activity in a long-term manner. NMDAR plasticity (e.g. NMDAR-LTP/LTD) has been described at several key brain areas where it strongly impacts spike transfer and could contribute significantly to learning and memory; however, very little is known about its molecular basis. Hippocampal mossy fiber-to-CA3 pyramidal cells (mf-CA3) synapses express robust bidirectional, burst timing-dependent NMDAR plasticity (BTD-NMDAR-LTP/LTD), which can be easily elicited with physiologically-relevant, coincident pre/postsynaptic burst activity. Both BTD-NMDAR LTP and LTD depend on postsynaptic calcium rise but the calcium sources differ, suggesting that different calcium dynamics could determine the bidirectionality of NMDAR plasticity. To test this hypothesis we combined electrophysiology with 2-photon laser microscopy in acute rat hippocampal slices in order to measure postsynaptic Ca^{2+} dynamics (i.e. calcium transients or CaTs) during the induction of BTD-NMDAR-LTP/LTD. We compared CaTs at thorny excrescences (TEs, the postsynaptic target of mf-CA3 synapses) with those generated at simple dendritic spines receiving associational/commissural (A/C) synapses which do not express NMDAR plasticity. We found that burst-induced postsynaptic CaTs associated with basal synaptic transmission at both TE and A/C spines are largely mediated by NMDARs, whereas CaTs evoked by back propagating action potentials are mostly mediated by L-type voltage-gated calcium channels. Intracellular stores and group-I metabotropic glutamate receptors (I-mGluRs) were also found to contribute to TE CaTs. Furthermore, CaTs were significantly larger during the induction of BTD-NMDAR-LTP than NMDAR-LTD, a difference that was also observed by direct postsynaptic activation using glutamate uncaging. Lastly, TE CaTs associated with BTD-NMDAR-LTP and LTD were differentially affected by pharmacological blockade of distinct Ca^{2+} sources. Together, our findings strongly suggest that the sign of NMDAR plasticity observed at mf-CA3 synapses (i.e. LTP/LTD) is critically determined by specific postsynaptic Ca^{2+} signals.

Disclosures: **S. Lutzu:** None. **K. Alvina:** None. **P. Castillo:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: Craig H. Neilsen (339705)

NYSDOH (Contract DOH01-C30836GG-3450000)

Title: Paired transspinal and transcortical associative stimulation modulates human spinal motor output

Authors: *M. KNIKOU, D. SANTORA, L. DIXON, M. M. IBRAHIM;
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Abstract: The neuronal networks of the human spinal cord translate inputs from multimodal peripheral receptors and descending motor tracts, and are prone to modulation and sustained plasticity. Paired associative stimulation (PAS) of presynaptic and postsynaptic cells produces spike-timing-dependent plasticity via synaptic mechanisms. However, the effects of pairing transspinal and transcortical stimulation on human spinal motor pathways are not known. In this study, we delivered paired transspinal and transcortical stimuli for 40-min at an interstimulus interval that TMS was delivered after (transspinal-transcortical PAS) or before (transcortical-transspinal PAS) transspinal stimulation in 14 healthy human subjects. In the transspinal-transcortical PAS, the transspinal stimulation served as the presynaptic input to cortical neurons. In the transcortical-transspinal PAS, transcortical stimulation served as the presynaptic input to spinal neurons. Spinal motor output was assessed via the transspinal evoked potentials (TEPs) recruitment curves and the amount of postactivation of TEPs. We found that PAS-induced plasticity at a spinal level can be achieved with pairing transspinal and transcortical stimulation. The effects depended on the timing between the two inputs since transcortical-transspinal PAS decreased and transspinal-transcortical had the opposite effect in the amplitude of knee/ankle TEPs recorded from both limbs at 0.2 Hz and at the same stimulation intensities before and after PAS. In contrast, from the TEPs recruitment curves we found that the amplitude of most ankle TEPs was decreased after transcortical-transspinal PAS and after transspinal-transcortical PAS. The decreased amplitude of TEPs coincided with increased gain of the spinal motor output system only after transcortical-transspinal PAS and not after transspinal-transcortical PAS. These findings provide evidence towards a new paradigm to alter spinal motor output in humans.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: JSPS Fellowship (DC2)

KAKENHI No15H04265

Title: Optimal learning with redundant synaptic connections

Authors: *N. HIRATANI[1,2], T. FUKAI[1,3];
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Abstract: Recent experimental studies suggest that, in cortical microcircuits of the mammalian brain, the majority of neuron-to-neuron connections are realized by multiple synapses. For instance, in the barrel cortex of juvenile mice, mean number of synapses per connection is estimated to be around 10. However, little is known on the functional benefit of having such redundant synaptic connections. Here, we show that redundant synaptic connections enable near-optimal learning in cooperation with synaptic rewiring. By constructing a simple dendritic neuron model, we demonstrate that, in multi-synaptic connections, synaptic plasticity approximates a particle-filtering algorithm, and wiring plasticity corresponds to its resampling process. The derived synaptic plasticity rule reconciles with dendritic position dependence of spike-timing-dependent plasticity observed in previous experiments. In particular, our study reveals a functional merit of anti-Hebbian plasticity at distal synapses. The model also explains why two synapses projected to different dendritic branches from the same axon seldom show spine-size correlation, while those on the same branch exhibit tight size correlation. The proposed framework is extensible for a detailed single neuron model, and also applicable to recurrent circuit models. In conclusion, our study provides a novel conceptual framework for synaptic plasticity and rewiring by focusing on redundancy in synaptic connections.

Disclosures: **N. Hiratani:** None. **T. Fukai:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: MRP Grant (2015R1A2A2A01004185) from Korean NRF

Title: Anti-Hebbian learning of optimal homeostatic IPSG amplitude and decay time

Authors: *J. K. KIM, C. D. FIORILLO;
Bio and Brain Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: For spikes to be sensitive to synaptic excitation requires properly balanced inhibition. We previously proposed that perfect homeostatic balance is achieved when the peak of an excitatory postsynaptic potential (EPSP) reaches exactly to spike threshold, so that the slightest variation in the amplitude of synaptic excitation determines whether or not a spike is generated (Fiorillo et al., 2014). With this as the homeostatic ideal, we used computer simulations and a measure of 'distance from optimality' to find the optimal amplitude and decay time of inhibitory postsynaptic conductance (IPSG) across a range of excitatory postsynaptic conductance (EPSG) frequencies of 5 to 800 Hz (with delay from EPSG to IPSG onset of 1.0 ms). With increasing frequency, the optimal ratio of IPSG to EPSG peak amplitude increased from 0.4 to 3.7, and optimal IPSG decay time constants decreased from 24 to 1.8 ms. According to theory, synapses and ion channel subtypes that are near to optimal in maintaining homeostasis can be selected through anti-Hebbian, spike-timing dependent learning rules (Fiorillo, 2008; Fiorillo et al., 2014). We tested this using NEURON software to create a single-compartment model with 9 synapses, each having a distinct decay time constant for synaptic inhibition (1.5 to 50 ms). Once learning resulted in stable synaptic weights, both IPSG amplitude and decay time closely matched those found to be optimal. To compare our estimates of optimality to experimental observations, we estimated typical EPSG input rates of 5 to 600 Hz across 19 types of neurons, and we found measures of IPSG decay time constants for each type of neuron (1 to 50 ms). We compared our theoretically optimal relation between decay time and EPSG input rate to the experimentally measured relation and found a highly significant quantitative match. Thus the theory of 'predictive homeostasis' appears able to predict and explain IPSG decay time. It also provides a means by which a neuron could learn IPSG decay times that are optimal with respect to the specific pattern of EPSG that it receives.

Disclosures: J.K. Kim: None. **C.D. Fiorillo:** None.

Poster

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Topic: B.08. Synaptic Plasticity

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Title: Activity-dependent anti-Hebbian glutamatergic spike-timing dependent plasticity within the lateral habenula

Authors: *L. D. LANGLOIS[1], F. NUGENT[2];
[1]Pharmacol., Uniformed Services Univ. of the Hlth. Scienc, Bethesda, MD; [2]Pharmacol., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: The lateral habenula (LHb) is an epithalamic structure involved in brain reward processes by providing negative reward signals to brain dopamine and serotonin systems. Hyperactivity of LHb neurons has been associated with exposure to addictive drugs and depression. One of the main risk factors for such mental disorders is early life stress-induced dysregulation of midbrain dopamine signaling. Our recent work has linked epigenetic modifications and metaplasticity within the ventral tegmental area (VTA) dopamine system to a single episode of maternal deprivation (MD) as an early life stressor. We have now extended our studies to the LHb and found that LHb neurons of MD rats (P21-P30) are also hyperexcitable. To address whether MD triggers changes in LHb excitability and DA signaling through induction of metaplasticity in the LHb, we started to investigate the induction of glutamatergic spike-timing dependent plasticity (STDP) of LHb neurons in response to near-coincident pre- and post-synaptic activities. Coincident pre and postsynaptic firing is required for glutamatergic STDP of LHb neurons as pre or postsynaptic activity alone is insufficient to induce plasticity. Neither neurons displaying silent, tonic nor irregular firing pattern expressed bidirectional STDP in response to all STDP induction protocols with different time intervals tested so far. Tonic firing neurons showed long-term potentiation (LTP), regardless of spike-timing order. Instead, in response to our spike-timing protocols, quiet and irregularly firing neurons either expressed LTD or no plasticity. This suggests that LHb neurons display activity-dependent anti-Hebbian STDP. Interestingly, the basal excitability of LHb neurons may reliably predict the direction of plasticity in STDP of the LHb. We will continue to characterize STDP and evaluate the effects of MD on STDP of LHb since MD-induced synaptic abnormalities and alteration in dopamine signaling from the VTA may be downstream to metaplastic changes of STDP within the LHb.

Disclosures: L.D. Langlois: None. **F. Nugent:** None.

Poster

401. Spike-Timing Dependent Plasticity

Location: Halls B-H

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Program#/Poster#: 401.08/G6

Topic: B.08. Synaptic Plasticity

Support: DARPA (N66001-14-C-4016)

NSF INSPIRE

NIH U01 (GM104604)

Title: Identification of a functional spike-timing-dependent plasticity rule from ensemble hippocampal spiking activity with generalized multilinear modeling

Authors: *B. S. ROBINSON[1], D. SONG[1], R. E. HAMPSON[2], S. A. DEADWYLER[2], T. W. BERGER[1];
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Abstract: The degree to which spike-timing-dependent plasticity (STDP) underlies fluctuations in synaptic strength during behavior is not well understood. One reason for this is that measuring how synaptic strength is influenced by spontaneously occurring behavioral spiking activity is experimentally challenging. Here, nonlinear dynamical modeling is used to identify functional connectivity strength between neurons by quantifying the conditional firing probability of an output neuron's spiking activity given an input neuron spiking event. This nonlinear dynamical model additionally identifies an STDP rule that characterizes how the relative input-output neuron spike timing relates to fluctuations in functional connectivity strength over time. Basis function expansion and generalized multilinear modeling are used to formulate and identify STDP model functions from recorded spiking activity in CA3 and CA1 hippocampal regions. The identified STDP models are able to capture weight fluctuations in functional connectivity strength for certain behavioral sessions. We also investigate how several sets of assumptions in STDP rule formation vary in ability to capture the dynamics of the observed spiking activity, such as weight-dependence and incorporation of triplets. The identified plasticity rules provide insights into how an STDP rule may lead to fluctuations in functional connectivity strength observed during behavior.

Disclosures: **B.S. Robinson:** None. **D. Song:** None. **R.E. Hampson:** None. **S.A. Deadwyler:** None. **T.W. Berger:** None.

Poster

401. Spike-Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: JSPS KAKENHI Grant Number 15H05877

Title: Potential roles of intracellular calcium dynamics regulated by calcium stores for spatial association of synaptic plasticity

Authors: D. FUTAGI, *K. KITANO;
Ritsumeikan Univ., Kusatsu, Japan

Abstract: Many studies have reported that the strength of a synapse undergoes a change depending on firing rates or spike timings of pre- and postsynaptic neurons. Furthermore, recent findings showed that synaptic plasticity induced at a synapse is accompanied by changes at the adjacent sites, namely, hetero-synaptic plasticity. It is strongly suggested that this collective synaptic plasticity should play an important role for forming and maintaining synaptic connectivity for neural functions whereas the mechanism that associates homo-synaptic plasticity with hetero-synaptic plasticity is not much known. Because spatiotemporal intracellular calcium dynamics mediated by calcium stores is likely to underlie the spatial association between the homo- and hetero-synaptic plasticity, it should be an important issue to reveal how such calcium dynamics is involved in the collective synaptic plasticity. In the present study, we studied a potential role of the calcium dynamics by numerical simulation of a computational model. Our model was built by combining a multi-compartmental neuronal model with a model of intracellular calcium dynamics, which enable us to see how a calcium increase triggered by pre- and/or postsynaptic spikes is regulated by the store dynamics. We here considered two different types of receptors on calcium stores that regulate calcium release: ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP3Rs). Model simulations showed these receptors differently function; RyR-mediated calcium increase was independent of pre- and postsynaptic spike timings and the distance from an induction site whereas IP3R-mediated one depended on them. In particular, IP3R could shape the temporal window known for the spike-timing-dependent plasticity. Thus, our results suggested that calcium release from the calcium stores would be involved in the spatial association of synaptic plasticity and the two different types of receptors could contribute to it differently.

Disclosures: D. Futagi: None. **K. Kitano:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: MEXT-Supported Proggram for the Strategic Research Fundation at Private Universities, 2013-2017

JSPS KAKENHI Grant Number 23500186

Title: Cholinergic modulation on LTD in rat hippocampal network

Authors: *E. SUGISAKI[1], Y. FUKUSHIMA[2], S. FUJII[3], N. NAKAJIMA[1], T. AIHARA[1]; [1]Tamagawa Univ. Grad. Sch. of Engin., Tokyo, Japan; [2]Kawasaki Univ., Kurashiki, Japan; [3]Yamagata Univ., Yamagata, Japan

Abstract: Synaptic plasticity is a fundamental property of learning and memory. The spike timing-dependent plasticity (STDP) protocol is one of the various stimulation that can induce synaptic plasticity. In interneuron-activated network, long-term potentiation (LTP) and long-term depression (LTD) can be observed by positive timing protocol (EPSP then BPAP) application depending on the relative timing of spikes, while only LTD can be induced by negative timing protocol (BPAP then EPSP) application. In a meantime, acetylcholine (ACh) is known as a crucial modulator for sensory inputs. The cholinergic neurons are projecting to pyramidal neurons and interneurons, and muscarinic ACh receptors (mAChR) and nicotinic ACh receptors (nAChR) are widely distributed in hippocampus. In the previous studies, the effect of each AChR activation on synaptic plasticity was investigated, however the combined effect of AChR activation is still unclear.In order to evaluate the combined influence of mAChRs and nAChRs on pyramidal neurons and interneurons on STDP in interneuron-activated network, patch clamp recording was made in the soma of pyramidal neuron applying a positive or negative timing protocol in the presence or absence of eserine (a cholinesterase inhibitor) using rat hippocampal slices. Moreover, the dependence of AChR-type specificity on STDP was investigated by the application of atropine or mecamylamine, mAChR or nAChR blocker respectively.As the results in interneuron-activated network, LTD observed by the positive timing protocol was switched to LTP, and the LTD by negative timing protocol was shifted toward potentiation in the presence of eserine. If nAChR and interneuron were prevented for only mAChR on pyramidal neuron to be activated, the larger STDP than that of the control condition, but smaller than that of the eserine application was observed at positive or negative timing protocol in interneuron-blocked network. On the other hand, when only nAChR on pyramidal neuron was activated, smaller STDP than the one of mAChR activation on pyramidal neuron was induced. Next in interneuron-activated network, the activation of mAChR on interneuron switched the STDP direction into LTD in the

absence of nAChR. Meanwhile, the activation of nAChR on interneuron did not change the direction of STDP. These results suggest that the STDP is shifted toward potentiation in the presence of ACh by co-activating mAChR and nAChR not only on pyramidal neuron but also on interneuron. Furthermore, the direction of STDP is decided by the combined activation of mAChR on pyramidal neuron and interneuron, while nAChRs sensitively fine-tune the magnitude of STDP.

Disclosures: **E. Sugisaki:** None. **Y. Fukushima:** None. **S. Fujii:** None. **N. Nakajima:** None. **T. Aihara:** None.

Poster

401. Spike-Timing Dependent Plasticity

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Support: BFU2009-10034 MICINN (Spain)

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Title: Developmental profile of spike timing-dependent plasticity at CA3-CA1 synapses of mouse hippocampus

Authors: ***A. RODRIGUEZ-MORENO**, Y. ANDRADE-TALAVERA, P. DUQUE-FERIA; Univ. Pablo De Olavide, Seville, Spain

Abstract: Spike Timing-Dependent Plasticity (STDP) is a strong candidate for a synaptic mechanism involved in development and in learning and memory. The goal of this work was to determine the developmental profile of STDP in the hippocampus. We performed experiments in the CA1 region of hippocampal slices prepared from P4-P112 mice using the whole-cell configuration of the patch-clamp technique. To induce t-LTP, a pre-post pairing protocol (with the presynaptic activity occurring 5 ms before a postsynaptic action potential) was applied after a stable EPSP baseline period of 10 min. To induce tLTD, a post-pre pairing protocol (with the presynaptic activity occurring 18 ms after a postsynaptic action potential) was applied. We found that a pre-post pairing protocol induced a robust t-LTP after the first postnatal week and at P21-P28, P65-P79 and P103-P112 mice. A post-before-pre pairing protocol was able to induce a significant depression at P8-P14 (72 ± 3%, n = 7) as well as P15-P21. However, this protocol was unable to induce t-LTD in P22-P42 mice. These results suggest that spike timing-dependent

depression emerges during the first postnatal week and disappears in the adulthood and that t-LTP emerges during the second postnatal week and persists in the adulthood at CA3-CA1 synapses.

Disclosures: A. Rodriguez-Moreno: None. **Y. Andrade-Talavera:** None. **P. Duque-Feria:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH

NSF

Title: Complex circuits from simple learning rules

Authors: *J. OLSON, G. KREIMAN;
Harvard Univ., Cambridge, MA

Abstract: Cortical circuits follow specific canonical connectivity patterns that are important for local computations. These canonical motifs have been described throughout cortex. What are the learning rules governing the cortical wiring instantiating canonical circuits? While the broad strokes of circuit development generally rely upon molecular cues dictating the general targets of neuronal migration as well as the direction of dendritic and axonal growth patterns, the refinement and specification of circuits depend upon neural activity and plasticity mechanisms. Here we ask whether simple learning rules alone can give rise to such complex circuitry. We focus on spike timing dependent plasticity (STDP), where a synapse is strengthened or weakened based on the relative timing of spiking between the pre- and post- synaptic neurons. We use a computational model to show how a combination of two different variations of STDP, classical STDP (cSTDP) and reverse STDP (rSTDP), can generate canonical circuits. Starting from all-to-all connectivity, the model dynamically converges onto a canonical circuit resembling the cortical columnar circuit described in macaques by Lund (Lund, 2002) and in cats by Douglas and Martin (Douglas and Martin, 2004). The configuration of STDP rules necessary for the model to converge to the canonical circuit is consistent with experimental observations. The target circuit motifs arise if and only if synapses from layer 2/3 to layer 5/6 follow either cSTDP or rSTDP as reported by Letzkus et. al. (Letzkus et. al. 2006) and synapses from layer 4 to layer

2/3 follow only cSTDP as reported by Feldman (Feldman 2000). Our results make further predictions about the STDP rules to be expected among different cortical layers. In summary, the current model demonstrates that simple activity-dependent plasticity rules can give rise to the type of complex connectivity patterns that are pervasive throughout neocortex and provides a principled path to explain connectomics data.

Disclosures: J. Olson: None. **G. Kreiman:** None.

Poster

401. Spike-Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Title: Experience-dependent regulation of spike-timing dependent plasticity of inhibition in auditory cortex

Authors: *E. D. VICKERS[1], R. SCHNEGGENBURGER[2];
[2]BMI, [1]EPFL, Lausanne, Switzerland

Abstract: In the input layers of tonotopically organized auditory cortex (A1), feedforward inhibition from parvalbumin interneurons (PV INs) onto principal cells (PC) plays a critical role in controlling the window of temporal integration of thalamic inputs. In this feedforward circuit, the temporal relationship between action potentials (APs) of PV INs and PCs is precise; spike-timing dependent plasticity (STDP) of inhibition might therefore allow for activity-dependent regulation of PC integration and AP generation. Here we characterized STDP of PV IN GABAergic output synapses in the input layer of A1 by making paired whole-cell recordings between genetically identified PV INs and PCs, initially at P15 - P22. The STDP induction protocol consisted of 50 pairings of single pre- and postsynaptic APs at 0.2 Hz. We observed robust bi-directional STDP of inhibition. Long-term potentiation of inhibition (iLTP; ~55%) followed post-then-pre AP pairings (dt ~ -5 ms), and long-term depression of inhibition (iLTD; ~30%) followed pre-then-post AP pairings (dt ~ +5 ms). Variance-mean analysis suggested that iLTP has a presynaptic locus of expression. iLTP required the activation of L-type Ca2+ channels and localized postsynaptic Ca2+ transients (sensitivity to BAPTA); pharmacology showed a role for BDNF signaling via TrkB receptors. iLTP is therefore likely mediated by retrograde release of BDNF from PCs. iLTD also depended on TrkB signaling, but had a post-synaptic locus of expression.
We found that iLTD in young mice (P15-22) was transformed into iLTP with postnatal development (P28-31). This suggested that iLTD could be involved in experience-dependent A1

tonotopic map plasticity during a critical period, the closure of which coincides with a changed learning rule at inhibitory synpases. We developed an ex vivo slice approach, using "tetTag" mice, to visualize and record from neurons in A1 of mice that underwent 3 days (P11-14) of sound exposure (30 kHz, ~70 dB). This was followed by a brief "labeling" session (60 minutes at ~85 dB). 2-photon imaging of cFos promoter-driven tdTomato expression revealed sound-dependent tonotopic band labeling. We performed paired recordings between PV INs and tdt+ PCs (that is, PCs that were strongly activated by the 30 kHz sound labeling) and found that critical period sound exposure increased unitary IPSG amplitudes and converted iLTD into iLTP at P18 - P20. Taken together our work shows that bi-directional, BDNF-dependent STDP of inhibition is regulated by both experience and development. We hypothesize that spike-timing dependent iLTD is permissive for developmental plasticity of frequency tuning in A1.

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

Poster

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Topic: B.08. Synaptic Plasticity

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

Future Systems Healthcare Project of KAIST

Title: Spike-timing-dependent plasticity for short-term memory and long-term memory

Authors: *Y. PARK[1], W. CHOI[1,2], S.-B. PAIK[1,2];
[1]Bio and brain engineering, KAIST, Daejeon, Korea, Republic of; [2]Program of Brain and Cognitive Engineering, KAIST, Daejeon, Korea, Republic of

Abstract: Spike-timing-dependent plasticity (STDP) is a commonly observed learning rule that precisely modulates synaptic strength according to the temporal coherence of pre-and postsynaptic spike timings (Bi and Poo, 1998). Although STDP is considered to be a critical mechanism of learning and memory, how STDP can specifically modulate neural connections to form a various type of memory still remains unclear. Here we suggest that the profiles of STDP can determine the types of memory in learning – short-term or long-term. We propose an idea that short-term and long-term memories can originate from two distinct STDP rules: Asymmetric STDP (AS) (Gütig et al, 2003) and Symmetric STDP (SS). The main difference between AS and

SS is the symmetry of weight dependent learning rate profile: In the AS model, weak synapses are easily strengthened but hard to be weakened, while SS model makes both weak and strong synapses harder to change their synaptic weights compared to mid-range strength synapses.
To test our idea, we constructed a model feedforward neural network, consisting of two layers of leaky integrate-and-fire neurons; then we introduced random patterns of spike train to the networks as a simulation of information received. We defined memory as the ability of a system to retrieve consistent responses when trained pattern of spikes is introduced to the system repeatedly. Based on this definition, we confirmed that both AS and SS systems were capable of forming memories. However, the two systems showed noticeable differences in terms of memory sustainability and appendability: Networks with AS created memories that decayed relatively fast and was easily replaced by new information appended, while networks with SS formed memories that did not show any noticeable decay and persisted even when new information was added. Such characteristics of AS and SS networks appeared similar to the feature of short-term and long-term memories, respectively. Lastly, we tested a new learning rule, hybrid STDP, which is made from the linear combination of AS and SS kernels. Our simulation of hybrid STDP could generate memories with intermediate properties between short-term and long-term memories.
Overall, our result indicates that a small difference in synaptic learning rule may cause a significant difference in the performance of a memory system. Our study implies that the nature of short-term and long-term memory might not be completely different but share the same mechanism of memory formation with different learning rules.

Disclosures: **Y. Park:** None. **W. Choi:** None. **S. Paik:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: No 2014/15/N/NZ4/01912

Title: CD44 adhesion molecule involved in molecular mechanisms responsible for stabilization of dendritic spines

Authors: ***A. SKUPIEN**[1], M. ROSZKOWSKA[2], G. WILCZYNSKI[1], J. DZWONEK[1]; [1]Neurophysiol., Nencki Inst. of Exptl. Biol. Polish Ac, Warszawa, Poland; [2]Lab. of Cell Biophysics, Nencki Inst. of Exptl. Biol. Polish Acad. of Sci., Warsaw, Poland

Abstract: Dendritic spines are neuronal protrusions, each of which receives input typically from one excitatory synapse. Morphological changes in these actin-rich structures are associated with learning and memory formation. The loss or malformation of spines is also linked to many neurological diseases, which indicates the importance of proper regulation of spine morphology. Remodeling of actin cytoskeleton, the main structural component within dendritic spines, through actin binding proteins (e.g. cofilin), plays a key role in regulating spine structure and stability. It is also known that extracellular matrix (ECM) can regulate the process of synaptic plasticity. CD44 adhesion molecule is a receptor for hyaluronan, main extracellular matrix component in the brain. By its C-terminal domain, CD44 can interact with many intracellular proteins, including those engaged in actin cytoskeleton reorganization. In this study we report that CD44 adhesion molecule influences on dendritic spine morphology and modulates signaling pathways which control cofilin activation. Taking into consideration our pilot studies we hypothesize that CD44 adhesion molecule, focal adhesion kinase (FAK), LIM kinase and cofilin, belong to the same signaling module, which is responsible for regulation of dendritic spine stability.

Disclosures: **A. Skupien:** None. **M. Roszkowska:** None. **G. Wilczynski:** None. **J. Dzwonek:** None.

Poster

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Support: NCN Grant 2015/17/B/NZ4/02540

FNP Grant PARENT-BRIDGE/2011-3/2

NCN Grant 7873/B/P01/2011/40

Title: CD44, a novel synaptic cell adhesion molecule regulating structural and functional plasticity of dendritic spines

Authors: *J. DZWONEK[1], M. ROSZKOWSKA[2], A. SKUPIEN[1], T. WOJTOWICZ[4], M. KISIEL[4], B. RUSZCZYCKI[3], H. DOLEZYCZEK[1], J. W. MOZRZYMAS[4], J. WLODARCZYK[2], G. M. WILCZYNSKI[1];
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Abstract: Synaptic cell adhesion molecules regulate signal transduction, synaptic function, and plasticity. However, their role in neuronal interactions with the extracellular matrix (ECM) is still not well understood. Here, we report that the CD44, a transmembrane receptor for hyaluronan, modulates synaptic plasticity. The diminished expression of CD44 affected the synaptic excitatory transmission of primary hippocampal neurons, simultaneously modifying dendritic spine shape. The frequency of miniature excitatory postsynaptic currents decreased, accompanied by dendritic spine elongation and thinning. These structural and functional alterations went along with a decrease in the number of presynaptic Bassoon puncta, together with a reduction of PSD95 levels at dendritic spines, suggesting a reduced number of functional synapses. Lack of CD44 also abrogated spine head enlargement upon neuronal stimulation. Moreover, our results indicate that CD44 contributes to proper dendritic spine shape and function by modulating the activity of actin cytoskeleton regulators i.e. three small Rho GTPases (RhoA, Rac1, and Cdc42). Thus, CD44 appears to be a novel molecular player that is involved in the regulation of functional and structural plasticity of dendritic spines.

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Poster

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Topic: B.08. Synaptic Plasticity

Title: Dendritic spine changes associated with long-term synaptic plasticity in nigral dopamine neurons.

Authors: *M. KIM, M. PARK;
Sungkyunkwan Univ. Sch. of Med., Suwon-Si / Gyeonggi-Do, Korea, Republic of

Abstract: Glutamatergic synapses in midbrain dopamine neurons play a critical role in reinforcement learning and drug addictions. Very recently, we have reported that dopamine neurons in the substantia nigra pars compacta (SNc) have two morphologically and functionally distinct types of glutamatergic synapses on the same dendrite; spine synapses and shaft synapses. However, it is not clear whether the dendritic spines in dopamine neurons like other neurons

undergo synaptic plasticity. Therefore, we have explored whether the dendritic spines are synaptically plastic or not in nigral dopamine neurons, using high-resolution two-photon confocal microscopy and wholl-cell patch-clamp recordings in the TH-eGFP mouse midbrain slices. By consecutive focal glutamate uncaging pulses on the tip of spine heads or dendritic shafts, similar to physiological release of neurotransmitter glutamate, we found that glutamate stimulation above a certain level increased diameter of spine heads and excitatory postsynaptic potentials (EPSPs), that were maintained up to 40 minutes, suggesting the long-term potentiation (LTP). A NMDA receptor antagonist, CPP, blocked these spine enlargements, no significant spine growth occurred. Therefore, we demonstrate that these newly found dendritic spines in nigral dopamine neurons are also plastic and play important role in reinforcement learning and drug addictions in midbrain dopamine neurons.

Disclosures: **M. Kim:** None. **M. Park:** None.

Poster

402. Structural Plasticity I

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Swedish Brain Foundation (AJ)

Title: Localization and regulation of nogo receptor 1 and its partners.

Authors: *A. T. BRODIN, K. WELLFELT, G. SMEDFORS, E. ARVIDSSON, L. OLSON, T. E. KARLSSON;
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Abstract: Nogo receptor 1 (NgR1) together with multiple partners regulates neurite growth. It has been shown that NgR1 restricts plasticity during both development and adulthood. Neuronal activity downregulates NgR1, and the inability to downregulate NgR1 impairs long term memory. Further, overexpression of NgR1 differentially affects spine populations and dendritic structure, as well as response to cocaine sensitization in different brain regions. However, it is yet unknown whether NgR1 acts presynaptically, postsynaptically, or at both sites. Also, while it has been shown that NgR1 is downregulated by activity, there is much unknown about how other components of the NgR1 signalling system are affected, and over what timescale. Therefore we investigate the localization and regulation of NgR1 and its partners. By imaging primary hippocampal cultures containing tagged Nogo-system proteins using confocal and STED microscopy we will characterize the localization of NgR1 and its interacting molecules. We use in situ hybridization to chart the expression of Nogo-related proteins during development. To uncover the regulation of NgR1, we use in situ hybridization to characterize the expression profiles following kainic acid treatment. We will also verify our findings in vitro by charting the regulation of NgR1 and associated proteins in primary hippocampal cultures after pharmacological stimulation. Our results should contribute to the understanding of localization and regulation of Nogo signaling system components.

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Poster

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Topic: B.08. Synaptic Plasticity

Title: Structural plasticity of dendritic spines during long-term synaptic depression

Authors: *A. THOMAZEAU[1], M. BOSCH[2], S. ESSAYAN-PEREZ[1], M. F. BEAR[1];
[1]MIT, The Picower Inst. For Learning and Memory, Cambridge, MA; [2]Inst. for Bioengineering of Catalonia, Barcelona, Spain

Abstract: Structural modifications of dendritic spines are crucial processes mediating synaptic plasticity, and thus are considered possible structural correlates of learning and memory. The mechanisms underlying structural plasticity of dendritic spines during long-term depression (LTD) are poorly understood. It is also unclear whether those mechanisms are altered in some neurodevelopmental disorders such as Fragile X syndrome, the primary cause of genetically-based mental retardation. By using time-lapse two-photon fluorescence imaging combined with

electrophysiology, we visualized CA1 hippocampal dendritic spines to (1) determine the structural changes they undergo after induction of different forms of LTD, (2) investigate whether this structural plasticity is altered in a mouse model of Fragile X syndrome. We found that NMDAR-dependent LTD, but not mGluR-dependent LTD, is accompanied by a shrinkage of dendritic spines in control mice. We also found that this structural modification is dependent on *de novo* protein synthesis. In contrast, we found that in Fmr1 KO mice, even though the structural changes associated with LTD are intact, the spine shrinkage associated with NMDAR-dependent LTD is no longer dependent on *de novo* protein synthesis. These results further substantiate the importance of dendritic spine dynamic in the expression of LTD, which is known to be altered in Fmr1 KO mice. Therefore they could also be critically linked to the phenotypes of Fragile X syndrome.

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Topic: B.08. Synaptic Plasticity

Support: CONACYT 133178

Title: Intracerebroventricular administration of growth hormone induces neural morphological changes in the CA1 region of the dorsal hippocampus and the prefrontal cerebral cortex of adult rats

Authors: J. OLIVARES HERNANDEZ[1], *F. A. GARCÍA-GARCÍA[2], E. JUAREZ AGUILAR[1];
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Abstract: Growth hormone (GH) has different positive effects on the brain function. For example, GH administration improves learning and memory, regulates neurogenesis, myelin synthesis and axonal regeneration. In addition, GH also enhances expression of the AMPA and NMDA receptors that are associated with synaptic plasticity. Currently, there are no studies that examining the trophic effect of GH on neurons structure. Therefore, the aim of the present work was to examine the effect of the intracerebroventricular (I.C.V.) injection of GH on dendritic morphology in the pyramidal neurons of the CA1 region of the dorsal hippocampus and Layer III of prefrontal cortex (PFC) using Golgi-Cox stain method and its subsequent analysis by the Sholl

method. Adult male Wistar rats received a daily ICV injection of GH (120 ng) per seven days, being euthanized 21 days later. Our data shows that GH administration increased the total dendritic length in pyramidal neurons of CA1 region of the dorsal hippocampus ($p<0.001$), compared with control rats that did not receive the GH injection. In the same way, GH induced a significant increment in the total dendritic length in pyramidal neurons of the Layer III of the PFC ($p = 0.013$). Interestingly, the branch-order analysis also revealed an increase in the dendritic length of pyramidal neurons of the hippocampal CA1 region specifically from third to eighth orders in GH treated animals ($p < 0.001$), compared with neurons from control rats. The same effect was observed in Layer III of the PFC but only in the range of the third to sixth order ($p < 0.001$). Altogether, our results suggest the participation of GH in the synaptic plasticity through the regulation of the dendritic tree density.

Disclosures: J. Olivares Hernandez: None. **F.A. García-García:** None. **E. Juarez Aguilar:** None.

Poster

402. Structural Plasticity I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 402.07/G19

Topic: B.08. Synaptic Plasticity

Support: National Science Centre grant 2013/09/N/NZ3/00108

Title: Analysis of the specificity of MMP-9 inhibitor on the nectin-3 shedding upon neuronal stimulation

Authors: *E. REJMAK-KOZICKA, M. DZIEMBOWSKA, K. KALITA, L. KACZMAREK; Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Structural changes of dendritic spines occurring in response to synaptic activity are accompanied by changes in the connections between pre- and postsynaptic membrane. The strengthening and weakening of these contacts can be modulated by nectin-3 that is postsynaptic Cell Adhesion Molecule (CAM), which role to interact with presynaptic nectin-1 is well known. Previously we described that the increased nectin-3 proteolysis under chronic stress conditions correlates with the elevated MMP-9 activity in the rat hippocampal CA1 fragment. The nectin-3 proteolysis was also observed in other model of the strong excitation induced by the seizures triggered by kainic acid. Proteolytic cleavage of the nectin-3 results in the appearance of a 20 kDa nectin-3 derived fragment. The cleavage was inhibited by the MMP-9 inhibitor (inhibitor I). To test the connection between the nectin-3 shedding and activity of the MMP-9 *in vitro* we

performed experiments on the hippocampal cultures. The results also shown that this process depends on the activation of NMDA receptor and the presence of Ca^{2+} / calmodulin. Moreover, taking into account the possibility of non-specific activity of the applied inhibitor we experimentally demonstrated its specific activity towards MMP-9 by conjugating inhibitor I with biotin with following purification on the magnetic streptavidin beads. We analyzed the binding capacity of inhibitor I to the endogenous and exogenous MMP-9 by gel zymography. Due to the MMP-9 low brain expression level and its secretion on the synapse upon neuronal stimulation we decided to use the hippocampi from rats injected with kainic acid (10h after injection, 10 mg/kg) for our experiments. The activity of MMP-9 in the inhibitor-bound probe was detected. The inhibitor's specificity towards MMP-9 was supported by the absence of MMP-2 activity in this probe, which is another abundant in the brain metalloproteinase showing gelatynase activity. This results strongly confirm that inhibitor I is specific towards MMP-9 and this protease causes fragmentation of the nectin-3.

Disclosures: E. Rejmak-Kozicka: None. **M. Dziembowska:** None. **K. Kalita:** None. **L. Kaczmarek:** None.

Poster

402. Structural Plasticity I

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Program#/Poster#: 402.08/G20

Topic: B.08. Synaptic Plasticity

Support: BBSRC

AstraZeneca

Medical Research Council (MRC) UK

Title: Exploring the molecular mechanisms underlying rapid estrogenic modulation of cortical connectivity

Authors: *P. RAVAL[1], K. J. SELLERS[1], J. MUKHERJEE[2], N. J. BRANDON[2,3], D. P. SRIVASTAVA[1];
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Abstract: There is increasing evidence that the regulation of structure and function of neuronal circuits is an essential component of normal cognitive function and behaviour. Several studies

have demonstrated concurrent changes in connectivity between neurons during and following the acquisition of learned behaviours. Estrogens, particularly its biologically active form 17β-estradiol (E2), have repeatedly been illustrated to have powerful influences over cognitive function and behaviour, which is believed to be, in part, driven by estrogenic regulation of neuronal connectivity. Specifically, estrogens have consistently been illustrated to regulate dendritic spine dynamics and shape synapse structure and function. It is becoming clear that estrogens have two modes of actions: classically, they activate and translocate the 'nuclear' estrogen receptors (ER), alpha (ERα) and beta (ERβ), to the nucleus and exert effects on neuronal circuitry via gene transcription, which manifest over hours to days. Alternatively, estrogens can also act rapidly through non-genomic actions via intracellular signalling within minutes to hours. These rapid effects can result in the initiation of signalling pathways leading to a number of cellular events, many of which are independent of gene transcription such as: dendritic spine turnover/remodelling; protein/receptor trafficking; and local protein translation. However, the molecular and cellular mechanisms that underlie, and the ER(s) responsible for this rapid regulation of cognition, have yet to be fully elucidated.
As the remodelling of cortical connectivity is believed to be an essential component of cognitive function, we have focused on understanding how estrogens can regulate specific cellular events such as, spine remodelling and how target protein and receptor trafficking/expression and local protein translation contribute to this in primary cortical neurons. Employing a combination of confocal, super-resolution and live imaging, we have monitored dendritic spine turnover in response to acute E2 treatment and found that there is a transient increase within 30 min. Additionally, through imaging and biochemical assays such as, Fluorescent Canonical Amino acid Tagging (FUNCAT) and Surface Sensing of Translation (SUnSET) we have found that there is a biphasic increase of local protein translation interestingly mirroring the activation of specific pathways such as ERK1/2 and mTOR and an increase in PSD-95 expression levels. These data provide an insight into the signalling pathways and cellular events that potentially contribute to the effects of estrogen on dendritic spine plasticity.

Disclosures: **P. Raval:** None. **K.J. Sellers:** None. **J. Mukherjee:** None. **N.J. Brandon:** None. **D.P. Srivastava:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIMH R01 MH104227

NIMH R01 MH094449

NINDS R01 NS078791

NIEHS R01 ES018990

NIEHS F32 ES026872

Title: Early postnatal manganese exposure affects primary motor cortex development in adolescent mice

Authors: *C. E. MOYER[1], S. A. BEAUDIN[2], D. R. SMITH[2], Y. ZUO[1];
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Abstract: Developmental manganese exposure is associated with fine motor deficits in children, as well as in animal exposure models. The impaired fine motor control that develops in response to developmental manganese exposure is thought to result from nigrostriatal dopamine hypofunction and receptor disturbances in young and adult animals. However, it is not known whether developmental manganese exposure also impairs the structure and function of the primary motor cortex (M1). M1 plays an important role in fine motor control, and plasticity of excitatory synapses in M1 is associated with skilled motor learning in animals. Thus, the objective of the current study was to determine if developmental manganese exposure disrupts excitatory synapse plasticity in M1 of adolescent mice. Mice were orally exposed to manganese daily prior to weaning. Taking advantage of the sparse labeling of cortical layer V pyramidal neurons in *Thy1*-YFP-H line mice, and using *in vivo* two-photon microscopy, we longitudinally followed dendritic spines (yellow fluorescent protein (YFP)-labeled postsynaptic structures of presumptive excitatory synapses) on the apical dendrites of M1 layer V pyramidal neurons of adolescent mice (approximately one month old). Alterations of dendritic spines in M1 were observed during adolescence in mice that had been exposed to manganese prior to weaning relative to littermate controls. These findings suggest that early postnatal manganese exposure affects adolescent cortical excitatory synapse development in M1, and may impair M1 circuitry. Further studies are required to determine if the effects of early manganese exposure on synapse plasticity in M1 contribute directly to the fine motor dysfunction reported in animal models of developmental manganese exposure.

Disclosures: **C.E. Moyer:** None. **S.A. Beaudin:** None. **D.R. Smith:** None. **Y. Zuo:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: National Natural Science Foundation of China (31400942)

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Title: Physical exercise prevented the loss of spines and improved the ability of memory through the BDNF-TrkB signal pathway

Authors: *K. CHEN[1], C. LAI[3], M. TAN[2], L. HUANG[2], L. ZHANG[2], A. LI[2], W.-B. GAN[4], K.-F. SO[2], C. REN[2];
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Abstract: Dendritic spines are critical for learning and memory. It has been shown that the dynamic change of spines underlied higher cognitive functions. However, the mechanism governing such spine development is unclear yet. This study thus focused the mechanism of physical exercise on spines plasticity. We used two-photon microscopy to find that continuous forced exercise in mice could prevent the loss of spines in barrel cortex, and improve animal working memories in novel texture discrimination task. The exercise could also elevate the expression of BDNF in barrel cortex. Moreover, the application of TrkB inhibitor suppressed such beneficial effects of exercise on spine dynamics. In conclusion, we proposed that physical exercise modulates spine plasticity in barrel cortex via TrkB-BDNF pathway. This study provides new insights for intervention of neurodegenerative disease and mental illnesses.

Disclosures: **K. Chen:** None. **C. Lai:** None. **M. Tan:** None. **L. Huang:** None. **L. Zhang:** None. **A. Li:** None. **W. Gan:** None. **K. So:** None. **C. Ren:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: Pilot COBRE on Aging and Regenerative Medicine A50886G5 5P20GM103629-03 to RM

Startup funds from the School of Medicine to RM

Louisiana Board of Regents Graduate Research Fellowship LEQSF (2013-18)-GF-17 to RV

Title: Age-dependent alterations in dendritic spine dynamics in the somatosensory cortex following whisker stimulation

Authors: *R. L. VOGLEWEDE[1], A. R. DEWITT[1], E. H. TRIMMER[2], R. MOSTANY[1,2]; [1]Neurosci., [2]Pharmacol., Tulane Univ. Sch. of Med., New Orleans, LA

Abstract: Within the brain, stability and plasticity of synaptic connectivity are cooperative forces that operate in tandem during learning and memory processes. Previous research suggests that aged mice display an increased dendritic spine density coupled with an elevated spine turnover ratio within the Layer V pyramidal neurons of the primary somatosensory cortex when compared to young adult mice, indicating that although there may be a higher number of synaptic connections in this brain area of animals age, those synapses also appear to be less stable over time. Functional implications of this elevated turnover with regards to learning and plasticity has not been uncovered. The question remains: is this increase in turnover ratio a compensatory or a maladaptive mechanism that aged animals employ as they learn and form memories associated with sensory experience? To answer this question, in this study we explore the implications of less stable, hyperdynamic synaptic connections within the aged cortex on learning and memory as animals undergo a sensory experience.
To investigate this, we employed sensory manipulation via continuous whisker stimulation with a piezoelectric actuator at 8Hz for 10 minutes a day (a model shown to induce long-term potentiation) over 4 days. We performed chronic *in vivo* two photon imaging through a 4 mm cranial window of apical tuft dendrites of layer V pyramidal neurons within the primary somatosensory cortex of Thy1-eGFP-M male mice within two age sets: young adult (aged 3-5 months) and aged (18-22 months). Mice were imaged over 46 days spanning before, throughout, and following sessions of bundled whisker stimulation. Stimulation occurred from days 8 – 11. Imaging sessions occurred every 4 days (days 0 - 20, 38 - 46) to observe long-term changes in spine dynamics. Additional sessions were held every 24 hours during the peri-stimulation period

(days 6 - 16) to examine these changes with better temporal resolution.
Our results indicate no changes in dendritic spine density as a result of the whisker stimulation protocol in both age groups. However, we observe a significant, acute increase in the dendritic spine turnover ratio at both 4 day and 24 hour intervals following whisker stimulation in the young adult but not aged mice, suggesting that sensory experience-dependent formation and elimination of synapses is impaired in the latter group and an alternative mechanism for sensory learning and memory may be present within synapses as animals reach old age.

Disclosures: **R.L. Voglewede:** None. **A.R. DeWitt:** None. **E.H. Trimmer:** None. **R. Mostany:** None.

Poster

402. Structural Plasticity I

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Program#/Poster#: 402.12/G24

Topic: B.08. Synaptic Plasticity

Support: DFG Grant FOR 2143

Title: Parvalbumin-positive interneurons of the hippocampus show input-dependent structural plasticity

Authors: A. FOGGETTI[1], T. SCHIFFELHOLZ[1,2], *P. WULFF[1];
[1]Inst. of Physiology, Univ. of Kiel, Kiel, Germany; [2]Dept. of Psychiatry and Psychotherapy, Univ. of Kiel, Kiel, Germany

Abstract: Dendritic spines compartmentalize electrical and molecular signaling and on principal cells are recognized as fundamental components for both synaptic transmission and synaptic plasticity. Little is known about spines on inhibitory interneurons. We show that parvalbumin-positive GABAergic interneurons in the mouse hippocampus carry considerable densities of dendritic spines, which undergo input-dependent spatial re-organization in response to behavioural experience.

Disclosures: **A. Foggetti:** None. **T. Schiffelholz:** None. **P. Wulff:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: Psychiatric Research Trust

Medical Research Council (MRC) UK

Royal Society UK

Brain and Behavior Foundation (formally National Alliance for Research on Schizophrenia and Depression (NARSAD))

Title: Estrogen sensitive G-protein coupled receptor (GPER1) rapidly regulates dendritic spine turnover and PSD-95 dynamics.

Authors: *K. SELLERS[1], P. RAVAL[1], I. A. WATSON[1], T. DEEB[2], J. MUKHERJEE[2], F. ERLI[3], D. A. GADD[1], N. BRANDON[2,4], D. P. SRIVASTAVA[1];
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Abstract: In the mammalian forebrain, the majority of excitatory synapses form on dendritic spines. Changes in the number of dendritic spines are important for brain development, plasticity and the refinement of neural circuits. Psd-95, an abundant scaffolding protein in the postsynaptic density of excitatory synapses, interacts with various signalling and structural molecules tethering them into the postsynaptic density; here, it can play a central role in the processes surrounding both synaptic plasticity and maturity. Evidence from multiple studies have demonstrated that the changes in the structure and function of neuronal circuitry occurs during acquisition of learned behaviours. Multiple studies have shown that 17β-estradiol (E2), the major biologically active form can modulate cognition within a rapid time frame through changes in neuronal circuitry and regulation of dendritic spines. Furthermore, it is clear that concurrent with the action of E2 on modulation of dendritic spine numbers it is also able to recruit PSD-95 to these novel dendritic spines. While these effects have been attributed in the main to signalling via the estrogen receptors (ER) ERα and ERβ, recent studies have suggested that the newly identified E2 sensitive receptor, G-protein coupled estrogen receptor 1 (GPER1), may also mediate E2-dependent modulation of cognition. However, how cognition is modulated through GPER1 and what signalling pathways or cellular events are initiated is not clear.

There is an increasing body of evidence that suggests that GPER1 may induce dendritic spine plasticity in a rapid time frame. We have focused on elucidating the molecular mechanisms involved in the downstream regulation of this rapid dendritic spine remodelling in response to GPER1. Here we report using super resolution microscopy that GPER1 is present at a subset of synapses in excitatory cortical neurons where it forms nanodomains colocalising with PSD-95 in the postsynaptic density. We demonstrate that precise pharmacological activation of GPER1 leads to a rapid linear increase in dendritic spines which is dependent on the activation of specific signalling cascades. Concurrent with this we have monitored the spatiotemporal dynamics of synaptic proteins including Psd-95 in response to activation of GPER1. Additionally through biochemical assays we have found that this interaction between Psd-95 and GPER1 is vital for GPER1 to exert its responses in the refinement of neuronal circuitry. These data demonstrate that in cortical neurons, E2 signalling via GPER1, is capable of remodelling neuronal circuits by increasing the number of excitatory synapses.

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Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01DA034116

Title: Actin dynamics contribute to the storage of drug-associated memories in both sexes.

Authors: *E. J. YOUNG[1], G. RUMBAUGH[2], C. A. MILLER[1];
[1]Metabolism & Aging, Neurosci., [2]Neurosci., Scripps Res. Inst., Jupiter, FL

Abstract: Encountering drug-related environmental cues, even after long periods of abstinence, can trigger retrieval of deeply engrained associative memories capable of eliciting drug seeking behavior. Dendritic spines are thought to serve as a structural storage site for memories, with actin polymerization serving a critical role in encoding the memory. We have previously reported the unexpected finding that inhibiting direct actin depolymerization within the AMY results in the immediate and persistent disruption of methamphetamine (METH)-associated memories, independent of retrieval, with a concomitant reversal of AMY dendritic spine density to baseline levels. However, the same manipulation has no impact on fear or food reward

memories, which also rely on the AMY. These findings indicate that METH-associated memories are supported by actin dynamics that remain uniquely active long after consolidation, allowing for their selective, retrieval-independent disruption. Unfortunately, the therapeutic potential of a direct actin depolymerizer is limited because of the number of peripheral processes that critically depend on actin. In previous work, we found that nonmuscle myosin II (NMII) triggers spine actin polymerization by exerting mechanical force on actin filaments and more recently, we demonstrated that NMII can be safely administered systemically to disrupt drug seeking behavior in the same way that direct actin depolymerization does. Thus, NMII represents an attractive target for pharmacotherapeutic development to prevent relapse. However, an abundance of evidence indicates that sex differences exist in drug abuse, so any potential therapeutic should also be thoroughly investigated in females. Therefore, we determined the impact of NMII inhibition on memory maintenance and drug seeking behavior in females. Similar to what we observed with males, inhibiting NMII in the basolateral amygdala complex (BLC) prior to context-induced reinstatement of self-administration disrupted drug seeking behavior. This single treatment produced a persistent disruption of drug seeking behavior, lasting at least one month. The therapeutic potential of NMII inhibition in females was extended by successful disruption of a METH-associated memory (conditioned place preference), but not fear memory, following systemic NMII inhibition. The METH-associated memory loss was accompanied by a marked decrease in BLC spine density. While spine density and the corresponding memory was unchanged in fear memory animals following NMII inhibition. Together, this data further argues for the development of a small molecule inhibitor of NMII for the prevention of relapse.

Disclosures: E.J. Young: None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: ERC AdG LiPsyD

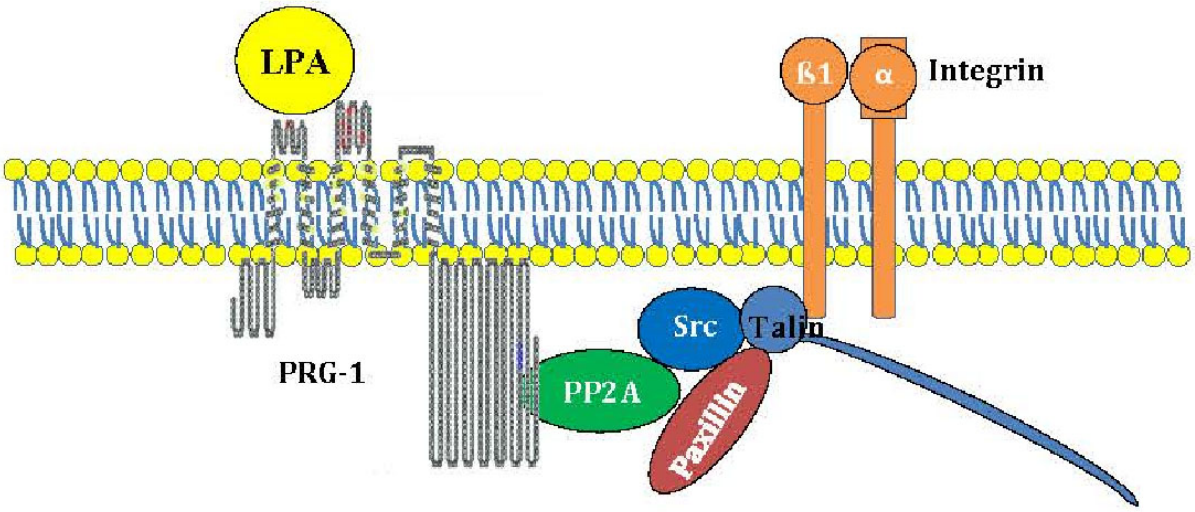
DFG CRC 1080

Title: PRG-1 regulates synaptic plasticity via intracellular PP2A/ITGB1-signaling

Authors: X. LIU[1], J. HUAI[1], H. ENDLE[1], L. SCHÜTER[1], W. FAN[1], Y. LI[1], S. RICHERS[1], H. YURUGI[1], K. RAJALINGAM[1], H. JI[1], H. CHENG[1], B. RISTER[1], G. HORTA[1], J.

BAUMGART[1], H. BERGER[1], G. LAUBE[2], U. SCHMIDT[1], M. J. SCHMEISSER[3], T. BÖCKERS[3], T. DELLER[4], A. VLACHOS[4], S. TENZER[1], *R. NITSCH[1], J. VOGT[1]; [1]Univ. Med. Ctr. Mainz, Mainz, Germany; [2]Charité, Berlin, Germany; [3]Univ. of Ulm, Ulm, Germany; [4]Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract: Alterations in dendritic spine numbers are linked to deficits in learning and memory. While we previously demonstrated that postsynaptic plasticity-related gene 1 (PRG-1) controls lysophosphatidic acid (LPA) signaling at glutamatergic synapses via presynaptic LPA-receptors, we now show that PRG-1 also affects spine density and synaptic plasticity in a cell-autonomous fashion via protein phosphatase 2A (PP2A)/ITGB1 activation. PRG-1-deficiency reduces spine numbers and ITGB1 activation, alters long-term potentiation (LTP), and impairs spatial memory. The intracellular PRG-1 C-terminus interacts in a LPA-dependent fashion with PP2A, thus modulating its phosphatase activity at the PSD. This results in recruitment of adhesome components src, paxillin and talin to lipid rafts and ultimately in activation of ITGB1. Consistent with these findings, activation of PP2A with FTY720 rescues defects in spine density and LTP of PRG-1-deficient animals. These results disclose a mechanism by which bioactive lipid signaling via PRG-1 could affect synaptic plasticity and memory formation.



Disclosures: **X. Liu:** None. **J. Huai:** None. **H. Endle:** None. **L. Schüter:** None. **W. Fan:** None. **Y. Li:** None. **S. Richers:** None. **H. Yurugi:** None. **K. Rajalingam:** None. **H. Ji:** None. **H. Cheng:** None. **B. Rister:** None. **G. Horta:** None. **J. Baumgart:** None. **H. Berger:** None. **G. Laube:** None. **U. Schmidt:** None. **M.J. Schmeisser:** None. **T. Böckers:** None. **T. Deller:** None. **A. Vlachos:** None. **S. Tenzer:** None. **R. Nitsch:** None. **J. Vogt:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: NWO VIDI #016.126.361 (CJW; HYH)

FOM #15PR3178 (DLHK)

Title: Dendritic coordination between excitatory and inhibitory plasticity

Authors: C. WIERENGA[1], D. KRUIJSSEN[1], *H. HU[1], B. RÓSZA[2];
[1]Univ. of Utrecht, Utrecht, Netherlands; [2]Two photon Imaging Lab. / Inst. of Exptl. Med. of the Hungarian Acad. of Sci., Budapest, Hungary

Abstract: For proper functioning of neuronal circuits during development and learning, changes in excitatory and inhibitory synapses need to be coordinated. Here we ask if excitatory and inhibitory plasticity is coordinated between nearby synapses within dendrites of CA1 pyramidal neurons.
We applied two-photon laser scanning microscopy to identify axo-dendritic crossings between dendrites of CA1 pyramidal cells and GABAergic axons in organotypic cultures of GAD65-GFP mice. Using two-photon glutamate uncaging, we activated several dendritic spines within ~10 μm of such crossings while the postsynaptic neuron was depolarized through a patch pipette to allow potentiation of the stimulated spines. We tracked possible morphological changes on the inhibitory axon at the crossing in response to the stimulation of nearby spines.
At 36% (12/33) of stimulated crossings, we observed a response of the GABAergic axon to the activation of nearby spines. In 42% of responding axons we observed a new inhibitory bouton appearing at the axo-dendritic crossing, while in the other cases the pre-existing inhibitory bouton at the crossing increased significantly in size and intensity. The induced inhibitory changes persisted for up to 40 minutes after the stimulation protocol. Without stimulation, axon intensity did not change during the imaging period (80 mins). Uncaging glutamate directly around a GABAergic axon did not induce bouton growth, indicating that the postsynaptic dendrite played an essential role in stimulating inhibitory bouton growth. We are currently analyzing how inhibitory changes are correlated with volume changes in stimulated spines.
Our results suggest that local mechanisms exist that monitor and regulate the local excitation-inhibition ratio within dendrites.

Disclosures: C. Wierenga: None. **D. Kruijssen:** None. **H. Hu:** None. **B. Rósza:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: NRF-2015R1D1A1A01060244

the National Honor Scientist Program of Korea

Title: Dissection of molecular mechanism of *Aplysia* Sec7 protein-induced neurite outgrowth.

Authors: Y.-W. JUN[1], J.-A. LEE[3], B.-K. KAANG[4], *D.-J. JANG[2];
[1]Kyungpook Natl. Univ., Snagju-si, Korea, Republic of; [2]Kyungpook Natl. Univ., Sangju-si/gyeongsangbuk-Do, Korea, Republic of; [3]Hannam Univ., Daejeon, Korea, Republic of; [4]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: ADP-ribosylation factors (ARFs) are small guanosine triphosphatases of the Ras superfamily involved in membrane trafficking and regulation of the actin cytoskeleton. *Aplysia* Sec7 protein (ApSec7), a guanine nucleotide exchange factor for ARF1 and ARF6, induces neurite outgrowth and plays a key role in 5-hydroxyltryptamine-induced neurite growth and synaptic facilitation in *Aplysia* sensory-motor synapses. However, molecular mechanism of ApSec7 functions was not clear. In the present study, firstly, we found that the coiled-coil domain of ApSec7 plays dual roles in intracellular targeting: efficient plasma membrane targeting through homodimer formation, and nuclear exclusion through either a CRM1-dependent or -independent pathway. Secondly, we found that ApSec7 could bind to active ARF6 and PI(3,4,5)P_3 and is localized to the plasma membrane, while another isoform, ApSec7(VPKIS) could bind to active ARF1 and PI4P and is localized to the Golgi complex. Thirdly, the activation of *Aplysia* ARF6 (ApARF6) as a downstream signaling of ApSec7 could induce neurite outgrowth in *Aplysia* sensory neurons. ApARF6-induced neurite outgrowth was inhibited by the co-expression of a Sec7 activity-deficient mutant of ApSec7 (ApSec7-E159K) via sequestration of active ApARF6 through the binding of the pleckstrin homology domain of ApSec7 at the plasma membrane. Thus, the results of the present study suggest that ARF6 signaling may be involved in downstream signaling of ApSec7-induced neurite outgrowth in *Aplysia* neurons.

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Poster

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Grant-in-Aid for Scientific Research on Innovative Area ‘‘Foundation of Synapse and Neurocircuit Pathology’’ from the Ministry of Education, Culture, Sports, Science and Technology of Japan

High-end Foreign Experts Recruitment Program of Guangdong Province

Title: A new optical method for rapidly erasing hippocampal synaptic memory.

Authors: *A. GOTO[1], K. MIYA[1,2], T. MATSUDA[3], T. NAGAI[3], Y. HAYASHI[1,4,5];
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Abstract: We previously found that in the initial phase of LTP, cofilin is transported to the spine, forms a stable complex with F-actin, persistently accumulates at the spine, and consolidates spine expansion. To spatiotemporally regulate cofilin activity during LTP, we introduced CALI (chromophore-assisted light inactivation) system, a technique to inactivate target proteins with light irradiation through reactive oxygen. For this purpose, we generated a fusion protein between cofilin and SuperNova (SN), a protein of GFP family that generates reactive oxygen upon light illumination. To validate this system, a persistent enlargement of the spine (structural LTP) was induced with two-photon (2P) uncaging of MNI-glutamate in the single dendritic spine in the hippocampal slice culture, which express SN-fused cofilin (CFL-SN), and 559 nm laser was subsequently irradiated. Followed by laser irradiation on the single spine 10 min after LTP induction, the spine volume gradually decreased. Decrease in the spine volume was also observed when laser was irradiated 20, 30 and 40min respectively after LTP induction. However decrease in the spine volume was no longer observed when laser was irradiated 50 min after LTP induction. Furthermore, the laser irradiation on the spine 1 min before sLTP had no effect on the spine enlargement. The laser irradiation on the spine without LTP induction had no effect on the spine volume. These results indicate cofilin is critical for maintenance of LTP up to 40 min after induction, and the inactivation of cofilin had negligible effect on LTP induction and the volume of unstimulated spine . To test system in memory formation, we expressed CFL-SN in hippocampal neurons by injecting AAV-floxed-CFL-SN

into CA1 region of CaMKII-Cre mouse, then implanted an optical fiber above the virus injection region. Memory was examined by Inhibitory Avoidance (IA) task. Memory was not impaired just by overexpress of CFL-SN in CA1 neuron. In contrast, memory was significantly impaired when 593 nm was irradiated on CA1 neurons expressing CFL-SN 2 min after an electrical shock at day 1. On the other hand, memory was not impaired when 593 nm was irradiated 1 min before the electrical shock. These data are consistent with those from slice culture. This new method can specifically erase memory shortly (2 min) after memory formation without any effect on subsequent induction of memory.

Disclosures: **A. Goto:** None. **K. Miya:** None. **T. Matsuda:** None. **T. Nagai:** None. **Y. Hayashi:** None.

Poster

402. Structural Plasticity I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 402.19/G31

Topic: B.08. Synaptic Plasticity

Title: The formation concept of protected areas of the human neocortex in focal damage

Authors: ***V. AKULININ**, A. MYTSIK, S. STEPANOV, V. RASUMOVSKY;
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Abstract: The study of neocortex compensatory and remedial changes in acute and chronic ischemia is an actual objective of neuromorphology. In this research, using histological, immunohistochemical neurons (NSE, Calbindin D28k, NPY), synapses (p38) and glial cells (GFAP) verification methods in morphometrical analysis, the focal neocortex changes were investigated in patients, who had a surgery for traumatic brain injury (n=5, biopsy) and brain tumor (n=25, biopsy). The patient's neocortex after clinical death (n=7, autopsy) was studied as well. 10 patients, died from accidental causes and served as controls. Morphometric image analysis of 25 fields of view for each case was performed using ImageJ 1.48, determines the area (μm^2) of fluorescent granule marker and number cells in sight. Statistical hypothesis testing was carried out used the program STATISTICA 8.0 with a nonparametric (ANOVA Friedman, Mann-Whitney, Wilcoxon, χ^2, frequency table, cluster analysis and multidimensional scaling) criteria. It is established that in different types of ischemia, comparing with the control, there were statistically significant differences between randomly selected neocortex fields of view in almost all the variables studied (total numerical density of neurons and astrocytes, immunopositive granules square, granules numerical density, the relative content of reactively changed neurons). In the control group the differences were observed mainly between the layers

(vertical stratification) of neocortex, while in ischemia these differences were between neighboring areas of every layer as well (vertical and horizontal changes stratification). The focuses of destructively changed neurons dominance around the fields of undamaged neurons were defined. The latter was manifested most clearly in chronic ischemia (tumors, postreanimation period). In comparison with the control, the following features were typical for these areas: 1) moderate deficiency of the total neurons numerical density (10-25%), 2) the dominance of normochromic neurons (50-70% of all neurons), 3) the high concentration of NSE-, Calbindin-positive neurons (110-140% of control group), 4) the increased content of NPY-positive material (120-200% of control group), 5) the proliferation of gliocytes and increased content of GFAP-positive material (120-140% of control group). We suggest that in chronic ischemia there are areas existing and forming around small focuses of destruction of the neocortex, that are protected from excite-toxic mechanisms of secondary ischemic damage.

Disclosures: V. Akulinin: None. **A. Mytsik:** None. **S. Stepanov:** None. **V. Rasumovsky:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: Scholarship 243297

Conacyt project 219847

Title: Enhancement of neuronal differentiation of mice olfactory epithelium stem cells by DNA methylation inhibition

Authors: *I. B. FRANCO ESTRADA[1], G. R. R. RAMIREZ-RODRIGUEZ[2], M. L. G. LAMAS GREGORI[3];
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Abstract: The olfactory epithelium is considered as a source of progenitors and stem cells with a high proliferation rate and neural differentiation capacity. Due to these features, olfactory epithelium cells (OECs) have been considered as possible tools for regenerative stem cell therapies in neurodegenerative diseases like Parkinson and Alzheimer. OECs differentiation

potential is acquired in response to intrinsic and extrinsic signals, which produce, among other effects, changes in DNA methylation and chromatin modifications. However, these mechanisms in mice OECs (mOECS) are not fully understood and the role of DNA methylation during their neuronal differentiation is still unknown. The aim of the present study was the *in vitro* characterization of mOECs so as to analyze their DNA methylation profile in order to evaluate the hypothesis that DNA methylation inhibition could improve their neuronal differentiation efficiency. The olfactory epithelium was isolated from five weeks old male Swiss Webster mice and cultured in DMEM-F12+10%SFB. On passages P2-P10 we analyzed qualitatively DNA methylation and demethylation-related genes (Dnmt1, Dnmt3a, Dnmt3b, Gadd45a and Gad45b) by RT-PCR and we show that mOECs maintain the expression of DNA methyltransferases in all the passages analyzed. Also we determined the DNA methylation (5mC and MeCP2) and hydroximethylation (5hmC) profiles by immunofluorescence. In order to induce neuronal differentiation, mOECs were exposed to retinoic acid and forskolin for 7 days. In these cultures we analyzed the morphology and expression of mature neuronal-associated genes (MAP2, NSE) by immunofluorescence. NSE was expressed in 69.5% on differentiated mOECs, whereas 35% were immunopositive to MAP2. We evaluated the effect of the pretreatment of mOECs with a specific DNMT1 inhibitor (Procainamide) before neuronal induction. Our results show increased levels of MAP2 mRNA expression in olfactory epithelium cells treated with 1.0mM of procainamide compared to the control. These results suggest that DNA methylation plays an important role during OECs differentiation and it may support their application for stem cell therapy.

Disclosures: I.B. Franco Estrada: None. **G.R.R. Ramirez-Rodriguez:** None. **M.L.G. Lamas Gregori:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: DFG FOR 2143

VW I/84236

BLBT 108 BIG

Title: Spines in parvalbumin-expressing interneurons undergo structural reorganization depending on behavioral experience

Authors: *D. KAUFHOLD, M. STRÜBER, M. BARTOS;
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Abstract: Dendritic spines provide electrical and biochemical compartments and are thus critical sites of synaptic transmission, synaptic plasticity as well as structural modifications of neuronal micro-circuits. In principal cells structural changes of dendritic spines correlate with long-term changes of synaptic input strength and memory formation. In contrast, GABAergic cells including perisoma-inhibiting parvalbumin-positive interneurons (PVIs) have been considered non-spiny. Recent studies showed behavior-dependent changes in protein expression levels in PVIs, structural remodeling of PVI axonal fibers and functional plasticity at their excitatory input synapses suggesting that DG PVIs may undergo structural and functional changes at their dendritic compartments. To address this open question we stereotactically injected an adeno-associated virus carrying the GFP reading frame inverted in a flip-excision cassette (AAV-FLEX-GFP) into the dentate gyrus (DG) of PV-Cre mice with subsequent detailed morphological analysis. We identified dendritic spines particularly in rodent DG PVIs. Here we asked whether PVI spines receive functional synaptic inputs and whether spine density can be altered in dependence on behavior.
We show that (1) hippocampal PVIs are indeed spiny but particularly those residing in the DG and not in CA3 and CA1; (2) about one third of DG PVIs are spiny with a maximal spine density of 0.57 ± 0.031 (SEM) spines/µm dendritic length; (3) PVI spines can be classified in stubby, thin and mushroom spines according to their size and shape; (4) unexpectedly, PVIs with somata at the hilus-granule cell layer border show higher spine densities than PVIs in the granule cell layer or close to the molecular layer; (5) PVI spine densities were higher in the dorsal DG than in the ventral DG. By electron microscopy we show that spine heads receive putative glutamatergic and spine necks GABAergic synaptic inputs. 2-Photon Ca^{2+}-imaging further revealed that these inputs are functional. To examine whether dendritic spines are influenced by behavioral experience, we kept mice under conditions of enriched environment for 3 weeks. We observed a structural change in spines suggesting alterations of synaptic strength and connectivity. Thus, PVI dendritic spines are functional and undergo structural changes upon behavioral experience.

Disclosures: D. Kaufhold: None. **M. Strüber:** None. **M. Bartos:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: National Natural Science Foundation of China No. 91132726

National Natural Science Foundation of China No. 91232306

Title: Neural circuit rewiring in the frontal association cortex of social defeated mice

Authors: *T. XU, Y. SHU;
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Abstract: Chronic stress is associated with occurrence of many mental disorders accompanied by alterations in neural morphology. Growing evidence, derived mostly from studies in medial prefrontal cortex, suggest that prefrontal cortex is involved in the stress response. The dendritic reorganization in pyramidal neurons of the prefrontal cortex has been investigated *in vitro* intensely. However, information for dynamic rewiring in the prefrontal cortex induced by chronic stress is still incomplete due to the lack of study *in vivo*. In this work, we determined the effects of chronic social defeat stress on spine plasticity of the frontal association cortex in *Thy1*-YFP H line transgenic mice *in vivo* by two-photon imaging. Unexpectedly, we found lower spine formation rate but higher survival rate of newly formed spines in the frontal association cortex of defeated animals, which ultimately results in the significant increase of new spine number. These findings demonstrate that chronic stress can lead to the newly persistent spine gains in the frontal association cortex and indicate this kind of adaptive change may relate to a certain memory about stress experience.

Disclosures: T. Xu: None. **Y. Shu:** None.

Poster

402. Structural Plasticity I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 402.23/G35

Topic: B.08. Synaptic Plasticity

Title: PGE1 in liposomes containing antagonized dendritic spine loss and reduction of VEGF & VEGFR2 in hippocampus of diabetic rats

Authors: *M. C. MOSTALLINO[1], F. BIGGIO[2], V. LOCCI[2], L. BOI[2], M. L. MANCA[2], A. M. FADDA[2], G. BROTZU[3], G. BIGGIO[2,1];
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Abstract: Diabetes mellitus is a common metabolic disorder that can lead to neurological complications such as cognitive impairment and dementia. In recent years, different clinical studies have drawn a clear association between type 1 diabetes with alterations in learning and memory. These clinical findings are strongly supported by data obtained in a rat model of type 1diabetes showing an impairment in learning and memory function. Cognitive impairment seems to be associated with modification of hippocampal synaptic plasticity, neurogenesis, reduced dendritic spine densities and glutamate receptor binding/expression in rat brain and reduced hippocampal and cortical volumes in humans. Diabetes related impairments in cognition and neural circuits function could be the consequence of the progressive damage at vascular level. Prostaglandins are cyclic, oxygenated fatty acids that exert a potent positive action on vascular endothelium in many tissues. Given the prostaglandins undergo to regulation by the rapid action of different enzimes we included PGE1 into liposomes made with phosphatidilcholine and Poly-L-lysine. These liposomes (1μg/kg) were intraperitoneally administered (twice a week for three months) to rats in which diabetes was induced by streptozotocin injection (70 mg/Kg). The glycemia was checked one time a week and 1 UI insulin retard was administered. Control rats and diabetics rats treated with saline has been used as control. Rats were sacrificed after three months. The dendritic spines density and morphology, VEGF (vascular endothelial growth factor) and VEGF-R2 (tyrosine kinase receptor of VEGF) expression levels were evaluated in the hippocampus and frontal cortex. In addiction, morphology by histochemistry and apoptosis were studied in gastrocnemius muscle, lungs and kidneys. All these parameters resulted altered in diabetic rats treated with saline while they were similar to those of control animals in diabetic rats treated with liposomes containing PGE1. The results suggest that such treatment is able to antagonize the neurochemical consequences elicited by experimental. This treatment can be considered an efficaceous terapy to counteract the negative effects of diabete in different vascular districts, brain included.

Disclosures: **M.C. Mostallino:** None. **F. Biggio:** None. **V. Locci:** None. **L. Boi:** None. **M.L. Manca:** None. **A.M. Fadda:** None. **G. Brotzu:** None. **G. Biggio:** None.

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: AHA 14POST20490122

NIH RO1NS062736

Title: NMDA receptor signaling mechanisms in activity-dependent spine shrinkage.

Authors: *I. S. STEIN, K. ZITO;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: The modification of brain circuits in response to sensory stimuli and heightened synaptic activity is essential for learning and memory. The formation and retraction of dendritic spines contribute to this activity-dependent circuit refinement. Indeed, the pruning of excessive dendritic spines during development has been linked to improvements in behavioral performance. In addition, increased spine shrinkage and loss of dendritic spines, as seen in neurological brain disorders and following brain injury, is associated with cognitive deficits. What are the molecular mechanisms driving shrinkage and elimination of dendritic spines in response to neural activity? Morphological changes of dendritic spines are closely correlated with changes in synaptic strength. Spine shrinkage, like long-term depression of excitatory synaptic transmission (LTD), is driven by low-frequency glutamatergic stimulation and subsequent activation of the NMDA-type glutamate receptor (NMDAR). We have recently shown that NMDAR signaling can drive shrinkage of dendritic spines independent of ion flux through the NMDAR, and that activation of p38 MAPK is required for this non-ionotropic NMDAR-dependent spine shrinkage. Currently, we are using a combination of two-photon imaging and biochemical analysis to further investigate the downstream mechanisms involved in activity-dependent spine shrinkage and synaptic depression. Results from these experiments will lead to a better understanding of the mechanisms that drive the shrinkage and elimination of dendritic spines normally during neuronal circuit refinement and extensively in disease.

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

Poster

402. Structural Plasticity I

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Topic: B.08. Synaptic Plasticity

Support: BK Grant

Title: The RapGEF Gef26 regulates synaptic development via inhibition of BMP signalling

Authors: *K. HEO;
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Abstract: Synaptic development and plasticity are critically dependent upon transsynaptic retrograde signaling from postsynaptic cells to presynaptic terminals. At the *Drosophila* neuromuscular junction (NMJ), the BMP homolog Glass bottom boat (Gbb) is secreted from postsynaptic muscles and regulates the growth and function of presynaptic terminals. Here, we present evidence that Gef26, a guanine nucleotide exchange factor for Rap small GTPases, acts together with Rap1 to restrain synaptic growth of NMJs. Mutations in *gef26* cause NMJ overgrowth characterized by excessive satellite bouton formation. This defect is rescued by presynaptic, but not postsynaptic, expression of Gef26. Genetic interactions indicate that Rap1 is a major target for Gef26 in the regulation of synaptic growth, and provide evidence that synaptic overgrowth in *gef26* or *rap1* is caused by elevated BMP signaling. *gef26* and *rap1* NMJs display elevated levels of phosphorylated Mad (p-Mad), a readout of BMP signaling. Synaptic overgrowth in *gef26* and *rap1* is sensitive to the level of BMP signaling. Based on our data, we propose that GEF26/Rap1 signaling regulates synaptic development by inhibiting presynaptic BMP signaling. We are currently investigating the cellular mechanism underlying Gef26/Rap1 regulation of BMP signaling.

Disclosures: K. Heo: None.

Poster

402. Structural Plasticity I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 402.26/G38

Topic: B.08. Synaptic Plasticity

Title: Vortioxetine increases phosphorylation of GluA1 subunit of AMPA receptor and alters other molecules associated with neuroplasticity

Authors: *L. WESTRICH[1], J. WALLER[2], B. CASE-WHITESIDE[1], M. GULINELLO[3], C. SANCHEZ[2], Y. LI[2];
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Abstract: Neuroplasticity plays an important role in the regulation of brain functions, disruptions of which are associated with mood disorders and cognitive impairment. Neuroplasticity can be altered by neuroactive medications and by aging. Vortioxetine, a multimodal antidepressant, has shown positive effects on cognitive functions in both pre-clinical and clinical studies. In animal studies, vortioxetine was shown to regulate functional plasticity, such as long-term potentiation, and structural plasticity, such as dendritic branching and spine morphology via an increase in glutamate neurotransmission. Activation of calcium/calmodulin-

dependent kinase type II alpha (CaMKIIα) and one of its main targets, the AMPA receptor (AMPAR), are critical for glutamate neurotransmission-induced neuroplasticity and for learning and memory. In addition, molecules that control the trafficking of AMPARs, such as cofilin (an actin depolarizing factor), have an essential role in the formation and remodeling of spines and dendrites, thereby modulating neuroplasticity. Consequently, we investigated the expression and phosphorylation status of CaMKIIα and GluA1 subunit of the AMPAR in cultured hippocampal neurons acutely treated with vortioxetine and examined the effects of chronic vortioxetine on expression and phosphorylation status of cofilin and GluA1 subunit of AMPAR in cortical synaptosomes of young and middle-age mice. Dissociated rat hippocampal cells (obtained from fetuses at embryonic day 18 and cultured for 21 days) were treated with vortioxetine, nefiracetam (CaMKIIα activator) or control for 1 h. Young and middle-aged female C57BL/6 mice were treated with vortioxetine-containing or regular chow for 1 month. The levels of total and phosphorylated CaMKIIα, GluA1 subunit of AMPAR and cofilin were measured by Western blotting and vortioxetine's effects were analyzed by 2-way ANOVA followed by post-hoc Tukey-Kramer tests. Acute vortioxetine increased phosphorylation of CaMKIIα without any effects on the total CaMKIIα protein level. This was accompanied by an increase in phosphorylation of GluA1 subunit of AMPAR at serine 845 without affecting phosphorylation at serine 831 or the total GluA1 protein level. In young mice, chronic vortioxetine increased cofilin phosphorylation without altering total cofilin protein level, and increased phosphorylation of GluA1 subunit of AMPAR at serine 845 without altering serine 831 or total GluA1 protein level. Our results indicate that vortioxetine modulates molecular targets related to neuroplasticity, which may elucidate the beneficial effects in cognitive function observed in clinical studies.

Disclosures: **L. Westrich:** A. Employment/Salary (full or part-time): Lundbeck Research (part-time). **J. Waller:** A. Employment/Salary (full or part-time): Lundbeck Research (full-time). **B. Case-Whiteside:** A. Employment/Salary (full or part-time): Lundbeck Research (part-time). **M. Gulinello:** F. Consulting Fees (e.g., advisory boards); Lundbeck Research. **C. Sanchez:** A. Employment/Salary (full or part-time): Lundbeck Research (full-time). **Y. Li:** A. Employment/Salary (full or part-time): Lundbeck Research (full-time).

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

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Title: Phosphorylation status of eIF4B S406 is a molecular switch in BC RNA-mediated translational control

Authors: *T. EOM[1], I. A. MUSLIMOV[1], S.-C. CHUANG[1], R. K. S. WONG[1,2], H. TIEDGE[1,2]; [1]Physiol. and Pharmacol., [2]Neurol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Regulatory Brain Cytoplasmic (BC) RNAs repress translation at the level of initiation by interacting with eukaryotic initiation factors (eIFs) 4A and 4B. BC RNAs inhibit recruitment of the 40S small ribosomal subunit by directly competing with 18S rRNA for binding to eIF4B. However, the mechanism for reversible regulation of BC RNA-mediated translational control is not well understood. Electrophoretic mobility shift assays (EMSAs) indicated that phosphorylation of eIF4B at serine 406 (S406) increased the affinity of BC RNA binding. We observed that BC RNA-mediated translational control was modulated by the phosphorylation status of eIF4B S406. eIF4B S406 phosphorylation was significantly reduced upon stimulation with DHPG, an agonist of group I mGluRs, or bicuculline, an antagonist of $GABA_A$Rs. Mediated by protein phosphatase (PP) 2A, eIF4B S406 was dephosphorylated within less than 2 min following receptor activation. This dephosphorylation resulted in the release of BC RNAs from eIF4B and in a substantial increase of protein synthesis. Subsequent re-phosphorylation of eIF4B S406 enabled high-affinity binding of BC RNAs, causing translational repression. Our data indicate that the phosphorylation status of eIF4B S406 serves as a molecular switch in BC RNA-mediated translational control. It suggests a novel molecular mechanism that links neuronal activity to translational control.

Disclosures: T. Eom: None. **I.A. Muslimov:** None. **S. Chuang:** None. **R.K.S. Wong:** None. **H. Tiedge:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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Topic: B.08. Synaptic Plasticity

Support: MH091676

MH099799

EY011261

Title: Identification of the newly synthesized protein required for synaptic plasticity in Xenopus laevis

Authors: *H.-H. LIU[1,2], W. SHEN[1,4], L. SCHIAPARELLI[1], D. MCCLATCHY[3], J. R. YATES, III[3], H. T. CLINE[1,2,3];
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Abstract: Emerging evidence has shown the importance of newly synthesized proteins in long-term synaptic plasticity and memory formation. In an effort to uncover mechanisms of experience-dependent synaptic plasticity, we performed quantitative proteomic analysis using improved bio-orthogonal metabolic labeling (BONCAT), an unbiased method to label newly synthesized proteins, with mass spectrometric multidimensional protein identification (MudPIT) to identify candidates that undergo dynamic protein synthesis in response to visual conditioning in the visual system of *Xenopus laevis* tadpoles. From two independent experiments, we detect a large variety of proteins, which are annotated to multiple cellular organelles or compartments, and to many cellular functions, for example, proteasome 26S subunits, RNA-binding proteins and cytoskeleton proteins. This list of candidates, together with the follow up functional analysis, should help us uncover the molecular machinery participating in synaptic plasticity. In addition, we also used fluorescent noncanonical amino acid tagging (FUNCAT) to investigate the dynamic in local protein synthesis in response to visual conditioning and found that Fragile X mental retardation protein (FMRP), a negative regulator of protein translation, is important for local protein synthesis and is required to maintain the visual conditioning-induced behavioral plasticity.

Disclosures: H. Liu: None. **W. Shen:** None. **L. Schiaparelli:** None. **D. McClatchy:** None. **J.R. Yates:** None. **H.T. Cline:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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CBDR/Dept of Biotechnology/Govt of India

Title: Role of FMRP bound miRISC at the crossroads of NMDAR and mGluR signalling

Authors: ***P. M. KUTE**[1,2], N. NEELAGANDAN[1], S. GOSH DASTIDAR[1], S. CHATTERJI[1,3], R. MUDDASHETTY[1];
[1]Inst. for Stem Cell Biol and Regenerative Med., Bangalore, India; [2]SASTRA Univ., Thanjavur, India; [3]Natl. Ctr. for Biol. Sci. (NCBS), Bangalore, India

Abstract: Activity mediated protein synthesis is a critical component of synaptic plasticity. NMDAR and mGluR mediated signalling are key constituents of synaptic plasticity both of which modulate synaptic protein synthesis. But how translation is specifically regulated downstream of either mGluR or NMDAR and their potential cross talk at the level of protein synthesis is not understood. Core objectives of this study is to characterize the common translation regulatory mechanism downstream of mGluR and NMDAR and then identify the unique components of this machinery which provides specificity. Fragile X Mental Retardation Protein (FMRP) is a known translation modulator that responds to mGluR signalling, the lack of which causes fragile X syndrome (FXS). FMRP regulates the expression of its target mRNAs by recruitment of microRNA induced silencing complex (miRISC) which can be reversed to facilitate translation by mGluR stimulation. Here we propose that FMRP-miRISC complex also regulates the translation downstream of NMDAR signalling. The composition of miRISC and its interaction with FMRP determines the translation status and specificity for mGluR and NMDAR signalling. We have identified MOV10 (an axillary component of miRISC) as a key determinant of this function. In synaptoneurosomal (SNS) preparation from Sprague Dawley (SD) rat (P30) brain cortex, downstream of NMDAR or mGluR signalling we observed contrasting association of MOV10 with miRISC and polysome. In case of NMDAR signalling, MOV10 dissociates from miRISC as measured by quantitative immunoprecipitation and moves to polysomes (as measured by polysome profiling) indicating translation of target mRNAs; whereas in mGluR signalling, MOV10 dissociates from polysomes and moves into inhibitory complex (miRISC) implying inhibition of target mRNAs. Immunostaining in cortical neuronal culture also shows reduced co-localization of AGO2 and MOV10 on mGluR signalling, consistent with the immunoprecipitation results. Interestingly, in Fmr1 KO rat SNS, MOV10 is completely absent in polysomes and hence lacks the NMDAR and mGluR mediated shift, bestowing a crucial role on FMRP for both these signalling. This result is further supported by our work in Neuro 2a cells, where siRNA mediated knockdown of FMRP shows a significant decrease in association of MOV10 with AGO2 and also lack of MOV10 in translating polysomes. These results indicate that MOV10 depends on FMRP to be associated with inhibitory complex of miRISC or with translating polysomes. We hypothesize that the composition of FMRP bound miRISC determines translation response of specific subset of mRNAs to different synaptic signals.

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Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Whitehall

Searle

Pew

Title: Communication of pathway-specific circuit activity to the genome by dendritic translation of the immediate early gene Npas4

Authors: *S. BRIGIDI[1], P.-A. LIN[2], B. L. BLOODGOOD[1];
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Abstract: During learning, task relevant neurons must convert fleeting stimuli into stable modifications in connectivity and excitability that alter their output. Mechanisms that govern plasticity are executed over many time scales and the most enduring forms of plasticity require regulation of the genome. Inducible transcription factors (ITFs) are a subset of immediate-early genes that support learning by linking transient molecular signals with long-lasting changes in cellular function through activity-dependent changes in gene expression. Npas4, a bHLH-PAS domain ITF, is undetectable in quiescent neurons but is rapidly induced in response to membrane depolarization *in vitro* and learning-related sensory experiences *in vivo*. Experience-dependent Npas4 expression dramatically reorganizes inhibitory synapses along the somato-dendritic axis of CA1 pyramidal neurons (PNs), increasing somatic and decreasing dendritic inhibition. This suggests that Npas4 regulates neuronal functions immediately linked to excitation by recalibrating inhibition in discrete domains of the PN. However, the nature of excitatory signals that lead to Npas4 induction, and whether the genome is capable of differentiating between excitatory signals that originate from distinct regions of the PN remains unknown.
Here we identify two independent pathways that lead to Npas4 expression in CA1 PNs that have distinct kinetics and functional consequences. A one second train of action potentials (APs) is sufficient to induce the accumulation of Npas4 in the PN soma through a signaling pathway that requires Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, transcription, and translation. In addition, we identify a pool of Npas4 mRNA localized to PN apical dendrites that is rapidly translated in response to NMDA receptor signaling initiated by sub-threshold transmission at Schaeffer Collateral synapses. Npas4 protein produced in the dendrites is then trafficked to the nucleus where it regulates gene expression. Surprisingly, Npas4 forms distinct heterodimers with

other bHLH-PAS transcription factors in response to APs or synapse activation. As each heterodimer binds a preferred nucleotide sequence, we predict stimulus-specific heterodimers facilitate communication of pathway-specific information to the nucleus in order to regulate unique aspects of Npas4's cellular phenotype. Together, our findings suggest that Npas4 executes an excitation-to-inhibition transfer function that distinguishes APs from synaptic activity originating from a specific excitatory pathway, and represents a novel mechanism for communicating circuit-relevant information to the genome.

Disclosures: **S. Brigidi:** None. **P. Lin:** None. **B.L. Bloodgood:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Support: NIH Z01 ES100221

Title: Transcriptome profiling in hippocampal dendrites

Authors: ***S. FARRIS**, J. M. WARD, M. SAMADI, Y. WANG, S. M. DUDEK;
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Abstract: Several lines of evidence suggest that experience-dependent translation of mRNA in dendrites is required for the persistence of experience-dependent changes in the brain. This spatial restriction of mRNA to subcellular domains enables local regulation of protein synthesis in a synapse-specific manner. It is currently unknown whether different cell types express different complements of dendritic RNAs to regulate specific forms of synaptic plasticity. Synapses in hippocampal area CA2 differ from those in neighboring subregions in that they do not undergo typical long-term potentiation, a process that requires local protein synthesis. In fact, we found that even the maintenance of baseline synaptic transmission in CA2 may require dendritic protein synthesis, as translation inhibitors led to a decrease in postsynaptic responses in CA2, but not in CA1. These data suggest that local protein synthesis may play a critical role in gating synaptic plasticity in CA2 dendrites. To identify the RNA transcripts in CA2 and surrounding subregion dendrites (CA1, CA3 and DG), we used laser-capture microdissection on hippocampal sections from a mouse line that expresses green fluorescent protein in CA2. RNA was isolated from the cell bodies and dendrites of each subregion (N=3 adult male mice) and used to generate cDNA libraries for RNAseq. Libraries from each animal were eight-plexed and run on a single lane of the Illumina NextSeq500 instrument acquiring 100bp paired-end reads.

Reads were aligned to the mouse genome (mm10) using STAR. Differential gene expression was determined using DESeq2 and alternative splicing was assessed using DEXseq. Finally, Illumina NextBio analyses were used to identify enriched canonical pathways in dendrites.
We found that CA2 dendrites are enriched for unique mRNA transcripts compared to neighboring CA1, CA3 and DG. In particular, genes involved in lipid metabolism and the generation of energy were expressed at higher levels in CA2 dendrites than neighboring dendrites. In addition, plasticity-restricting mRNAs, such as *Pcp4, Rgs14, Ptpn5 and Necab2* were enriched in CA2 dendrites and validated using single molecule fluorescent in situ hybridization. Identifying molecules in CA2 dendrites may lead to novel therapeutic targets for disorders in which dendritic protein synthesis has gone awry, such as those in the autism spectrum.

Disclosures: **S. Farris:** None. **J.M. Ward:** None. **M. Samadi:** None. **Y. Wang:** None. **S.M. Dudek:** None.

Poster

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NIH Grant NS046769

Title: Beta-adrenergic receptors activation in conditional dendritic RNA transport

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Abstract: In neurons, RNA transport plays an important role in synapto-dendritic protein synthesis. We previously reported that regulatory Brain Cytoplasmic (BC) 1 RNA is constitutively transported to dendrites where it operates as a repressor of local protein synthesis. ID3 and ID4 elements are phylogenetically derived from BC1 RNA by retroposition, and we asked whether such elements convey targeting competence to mRNAs that carry them. ID3-tubulin and ID4-tubulin reporter mRNAs were microinjected into somata of sympathetic neurons in culture that had been stimulated using agonists of alpha- or beta-adrenergic receptors (phenylephrine or isoproterenol, respectively). Following activation of beta-adrenergic receptors, injected reporter mRNAs were targeted to dendrites. Dendritic transport was not observed in the

basal state or following alpha-adrenergic activation. As a representative of an endogenous mRNA carrying an ID3 element, we selected CLN2 (ceroidlipofuscinosis, neuronal 2) mRNA. This mRNA was not delivered to dendrites in the basal state. However, CLN2 mRNA was transported along the dendritic extent following beta-adrenergic (but not alpha-adrenergic) receptor activation. Induced dendritic CLN2 mRNA transport was blocked in the presence of beta-adrenergic antagonist propranolol. The combined data indicate that beta−adrenergic activation signals to dendritic RNA transport.

Disclosures: I.A. Muslimov: None. **H. Tiedge:** None.

Poster

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Title: The kinesin motor protein KIF5B regulates RNA trafficking and dendritic spine morphogenesis in hippocampal neuron

Authors: *H.-L. CHAN, J.-D. HUANG, K.-O. LAI;
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Abstract: Protein synthesis in neuron can occur locally near individual synapses of the postsynaptic neuron, which may serve to translate synaptic activity into the formation of persistent synaptic connections and mature dendritic spines. To achieve local protein synthesis, specific mRNAs must first be transported to the neuronal dendrites. The anterograde transport of selective cargoes, including mRNAs, protein complexes and organelles to distal dendrites, is carried out by the Kinesin Superfamily (KIFs) of molecular motors. Among them, the three members of the KIF5 family (KIF5A, KIF5B & KIF5C) are present in the ribonucleoprotein complexes (RNPs) that transport dendritic mRNAs. However, it is not clear whether individual KIF5 perform redundant or distinct functions in the transport of RNPs and the regulation of synapse structure. Here we performed time-lapse confocal imaging using the RNA dye SYTO 14

and the Mitotracker CMXRox to monitor the dynamics of RNPs in dissociated hippocampal neurons derived from wild-type or KIF5B heterozygous knockout mice. Consistent with previous studies, majority of the RNPs were stationary, and the motile RNPs exhibited discontinuous movement which was either oscillatory (bi-directional movement over short distances) or unidirectional (moving one direction over long distances). Interestingly, KIF5B heterozygous neurons contained significantly fewer stationary RNPs, and higher proportion of RNPs displayed unidirectional movement. We further found that knock down of KIF5B expression in rat hippocampal neurons using short hairpin RNA (shRNA) led to the loss of mature spines and a significant increase in the number of immature filopodia. These findings suggest that the kinesin KIF5B is crucial for the transport and docking of selective mRNAs, which may subsequently regulate the maturation of dendritic spines.

Disclosures: **H. Chan:** None. **J. Huang:** None. **K. Lai:** None.

Poster

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Title: A transcriptional program underlying homeostatic scaling

Authors: ***K. SCHAUKOWITCH**, A. L. REESE, S.-K. KIM, G. KILARU, J.-Y. JOO, E. T. KAVALALI, T.-K. KIM;
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Abstract: Homeostatic scaling allows neurons to maintain stable activity patterns by globally altering their synaptic strength in response to changing activity levels. Deficits in scaling have been seen in models of autism spectrum disorders and schizophrenia, implicating its importance in cognitive function. One mechanism by which this process occurs is through up- or down-

regulating surface AMPA receptors. Decreasing activity by blocking action potentials with the sodium channel blocker, TTX, leads to an upregulation in synaptic strength, as seen by increases in AMPA mediated mEPSCs. In the opposite way, increasing activity with the $GABA_A$ receptor antagonist, bicuculline, decreases mEPSCs. It was previously shown that the increase in mEPSC amplitude in response to TTX could be blocked by a transcription inhibitor, suggesting that transcription is necessary for the scaling response. However, very little is known about the genes whose transcription is directly regulated by activity suppression or the signaling mechanisms underlying the transcriptional control. Using RNA-Seq, we identified nearly 100 genes that were specifically upregulated in response to TTX. In particular, *Neuronal pentraxin-1* (*Nptx1*), previously shown to promote AMPAR clustering, was increased ~3 fold after 6h of TTX, and knockdown of this gene blocked the TTX-induced increase in mEPSC amplitudes. SRF is a key transcription factor in regulating *Nptx1* induction, and this induction is calcium-dependent, indicating the existence of an active pathway to control transcription. Taken together, our study defines a novel transcriptional program that is able to sense the absence of activity and coordinate the cell's response to globally increase synaptic strength.

Disclosures: **K. Schaukowitch:** None. **A.L. Reese:** None. **S. Kim:** None. **G. Kilaru:** None. **J. Joo:** None. **E.T. Kavalali:** None. **T. Kim:** None.

Poster

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Title: Npas4 is necessary for circuit homeostasis and plasticity in the mouse primary visual cortex

Authors: ***X. SUN**, S. F. COOKE, M. J. BERNSTEIN, R. W. KOMOROWSKI, M. F. BEAR, Y. LIN;
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Abstract: Homeostatic regulation of synapses is key to maintaining the excitatory and inhibitory (E/I) balance of cortical circuits, upon which cortical function and plasticity depend. However, critical molecular and synaptic components that give cortical circuits the ability to constantly adjust to changing levels of activity are not well characterized. Here we examine the importance of Npas4, an immediate-early gene that has been shown to be involved in activity-dependent

modulation of GABAergic synapses, for maintaining circuit homeostasis and visual plasticity. Npas4 is strongly induced by visual stimuli in the mouse primary visual cortex (V1). Conditional deletion of Npas4 rapidly and drastically disrupted E/I balance in V1 by reducing the inhibitory tone. Consistent with the importance of E/I balance in visual learning, disruption of the V1 network resulting from the loss of Npas4 led to a deficit in stimulus-selective response potentiation (SRP), an *in vivo* visual learning paradigm that depends on synaptic plasticity. Our findings demonstrate that the activity-dependent pathway downstream of Npas4 is required for homeostasis and plasticity in the mouse visual cortex.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NSF GRFP

Whitehall

Searle

Pew

Title: Experience-dependent transcriptional regulation of inhibition in the CA1 microcircuit

Authors: *A. L. HARTZELL[1], K. M. MARTYNIUK[2], G. P. HIGERD[2], B. L. BLOODGOOD[2];
[1]Neurosciences, [2]Biol. Sciences, Section of Neurobio., UCSD, San Diego, CA

Abstract: The hippocampus is tasked with converting fleeting neuronal activity from a novel experience into a stable memory representation. One consequence of transient elevated activity is the expression of inducible transcription factors (ITFs), which can initiate gene programs that execute widespread and long-lasting changes in cell function. NPAS4 is an ITF that orchestrates the reorganization of inhibitory synapses along the somato-dendritic axis of a pyramidal neuron (PN), reducing dendritic inhibition while concurrently recruiting inhibitory synapses to the soma. This remodeling can profoundly affect the computations performed by the cell, allowing for a more plastic dendritic environment while simultaneously tuning the output of the neuron.

However, the interneuron (IN) subtypes from which the reorganized synapses originate are not known. Among the more than 20 IN subtypes in the mouse hippocampus, parvalbumin (PV)- and cholecystokinin (CCK)- expressing basket cells innervate the perisomatic domain of CA1 PNs. Inhibition provided by PV and CCK basket cells gate PN output in a fast and linear, or slow and modulatory manner, respectively. Synapses arising from PV versus CCK basket cells can therefore profoundly regulate either the timing or "mood" of the CA1 microcircuit, but it is poorly understood how experience remodels these individual synapse subtypes. We sought to determine the identities of the INs whose synapses undergo activity-induced, transcriptionally-regulated reorganization downstream of NPAS4 expression, and examine how this circuit change affects activity and plasticity of the CA1 microcircuit.
Using *in vivo* genetic manipulations and *ex vivo* electrophysiology, pharmacology, and anatomical techniques, we demonstrate that novel sensory experiences specifically result in the recruitment of CCK-basket cell synapses to the PN soma in an NPAS4-dependent manner, while PV basket cell synapses remain unchanged. Furthermore, deletion of NPAS4 from CA1 PNs significantly reduces depolarization-induced suppression of inhibition (DSI), a form of short-term plasticity expressed at CCK basket cell synapses. This has the potential to regulate the excitability and plasticity of the CA1 microcircuit through Npas4-dependent changes in CCK-mediated disinhibition of PNs. Together, our results suggest that novel sensory experiences increase the influence of CCK INs on the CA1 microcircuit through an NPAS4-dependent transcriptional program to drive PN plasticity. Ongoing experiments are further exploring how this IN-specific, experience-dependent circuit reorganization can affect the computations performed by the CA1 microcircuit.

Disclosures: **A.L. Hartzell:** None. **K.M. Martyniuk:** None. **G.P. Higerd:** None. **B.L. Bloodgood:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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Title: Deconstructing the relationship between neural activity patterns and Npas4-mediated gene expression

Authors: *P.-A. LIN[1], G. S. BRIGIDI[2], B. L. BLOODGOOD[2];
[1]Neurosciences, [2]Biol. Sciences, Neurobio. Section, Univ. of California San Diego, La Jolla, CA

Abstract: During learning, transient sensory inputs are processed into persistent changes in neuronal function and circuit connectivity. Gene regulation by inducible transcription factors (ITFs) plays a key role in facilitating this transformation. In comparison to other ITFs, such as Fos and Egr1, Npas4 is unique due to its selective induction by Ca^{2+} influx via membrane depolarization and not by other common signaling molecules such as cAMP, neurotrophins, and growth factors. Additionally, behaviorally-driven expression of Npas4 results in a dramatic reorganization of inhibitory synapses that are made onto CA1 pyramidal neurons (PNs), simultaneously decreasing inhibition at the proximal apical dendrites while increasing inhibition at the soma. This led us to hypothesize that Npas4 may be able to convey the origins and dynamics of excitatory signals to the nucleus, resulting in the regulation of distinct sets of target genes and providing a potential mechanism for the opposing plasticity observed at different inhibitory synapse populations. Excitatory signals generated in PNs can range in magnitude from bursts of action potentials (APs) to subthreshold EPSPs. Additionally, EPSPs convey information from many efferent pathways that are organized spatially along the somatodendritic axis of the neuron. To determine if Npas4 conveys information to the nucleus about the origins or intensity of a depolarizing signal, we prepared acute hippocampal slices from wild type mice and delivered brief, focal electrical stimulation of either the alveus or laminarly-restricted efferents in the CA1 region. These stimulation paradigms, in combination with appropriate pharmacology, generate antidromic APs in CA1 PNs or pathways-specific EPSPs, respectively. The CA1 region from equivalently stimulated slices was then microdissected and pooled for chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) of Npas4-bound genomic sites. Our results indicate that Npas4 regulates different target genes in response to APs or synaptic transmission in the proximal apical dendrites. Next we sought to determine if Npas4 also conveys information regarding the number, frequency, or duration of APs produced by a CA1 PN. Using a custom-built LED array, we optogenetically induced precise patterns of APs while blocking excitatory synaptic activity. We then used a combination of electrophysiology, immunohistochemistry, and ChIP-Seq to analyze the relationship between distinct spiking patterns and Npas4-mediated gene regulation. Our results indicate a novel role for Npas4 in communicating the spatial and temporal characteristics of excitatory activity to the nucleus.

Disclosures: **P. Lin:** None. **G.S. Brigidi:** None. **B.L. Bloodgood:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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Title: The role of activity-induced DNA breaks in synaptic plasticity, learning, and memory

Authors: *R. MADABHUSHI[1], O. KRITSKIY[1], F. GAO[1], T. X. PHAN[1], S. YAMAKAWA[1], T. GILLINGHAM[1], R. RUEDA[1], J. D. JAFFE[2], L.-H. TSAI[1];
[1]Brain and Cognitive Sci., MIT, Cambridge, MA; [2]The Broad Inst. of Harvard and MIT, Cambridge, MA

Abstract: Neuronal activity triggers the rapid expression of immediate early genes that play important roles in experience-driven synaptic changes, learning, and memory. While immediate early genes are primed for rapid induction, the specific impediments to their expression under basal conditions, and the mechanisms that relieve these constraints are still poorly understood. Recently, we reported that activity-dependent stimulation of neurons triggers the formation of DNA double strand breaks (DSBs) in the promoters of a subset of immediate early genes, including *Fos*, *Npas4*, and *Egr1*. These activity-induced DSBs are generated by the type II topoisomerase, Topo IIβ, and we showed surprisingly that Topo IIβ-mediated DSBs facilitate the rapid induction of these aforementioned IEGs. Here, we utilized a conditional mouse model to delete *Top2b* in excitatory neurons in the forebrain of adult mice. We report that Topo IIβ activity is essential for the formation of neuronal activity-induced DSBs and IEG induction *in vivo*, and that mice lacking Topo IIβ show deficits in associative and spatial learning behaviors. In addition to this, we describe the mechanisms that regulate the formation of Topo IIβ-mediated DSBs in response to neuronal activity. Specifically, using quantitative mass spectrometry, we show that neuronal activity triggers the rapid dephosphorylation of Topo IIβ, and that Topo IIβ dephosphorylation stimulates activity-dependent DSB formation. Together, these results provide new mechanistic insights into the regulation of neuronal activity-dependent DSB formation, and elaborate the significance of activity-dependent DSBs in crucial neuronal functions, including synaptic plasticity, learning, and memory.
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Poster

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Title: Adult Notch signaling underlies the rewarding memory of alcohol in *Drosophila*

Authors: ***E. PETRUCCELLI**[1], M. FEYDER[2], R. MUSTER[2], N. LEDRU[2], K. R. KAUN[2]; [1]Neurosci., [2]Brown Univ., Providence, RI

Abstract: Alcohol use disorder is a chronic relapse disorder impelled by its cravings. Despite its global, financial and public health burden, available treatments are both limited and inadequate. Safe and effective treatment requires a comprehensive understanding of mechanisms underlying the persistent rewarding memories of intoxication that trigger alcohol cravings and drug seeking behaviors. *Drosophila melanogaster* are an ideal genetic model for studying alcohol-induced behaviors, learning and memory, and complex molecular pathways because of their genetic tractability and natural preference for alcohol. Recently, we demonstrated that flies display acutely aversive, but enduring appetitive memories for alcohol. These behaviors require proper dopaminergic signaling and Notch pathway regulation. We hypothesize that alcohol reward memory requires neuron specific Notch-induced transcriptional responses that alter dopamine-related gene expression. To explore this, we used spatiotemporal genetic tools, molecular analyses, and brain immunohistochemistry techniques. We found that adult Notch receptor expression was required for alcohol reward memory in the mushroom body, an associative central brain structure. Alcohol exposure resulted in Notch-induced transcriptional responses in the adult brain as revealed by a Notch reporter tool. Lastly, we investigated the possible targeting of genes associated with dopamine signaling by Su(H), the transcriptional regulator of canonical Notch signaling using ChIP-qPCR. Together with our knowledge of neuroanatomical circuitry and molecular networks, this research helps unravel neurogenic mechanisms leading to, and possible treatment of, alcohol addiction.

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Poster

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Title: Examination of stalled polysomes in protein-synthesis dependent long-term potentiation in hippocampal cultures.

Authors: ***K. GINZBERG**, M. ANADOLU, T. E. GRABER, W. SOSSIN;
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Abstract: Late long-term potentiation (L-LTP) differs from early LTP in its dependence on protein synthesis. This dependence begins soon after LTP induction and may, similar to mGLUR-LTD (Graber et al., 2013), depend on fast local protein synthesis that is independent of translation initiation, resulting from the reactivation of stalled polysomes. There is evidence L-LTP requires some translation initiation (Graber et al, 2013), but this does not rule out additional requirements for proteins produced from stalled polysomes as well. In the stalled polysome model, the rate-limiting step of translation (initiation) occurs before mRNA transport, and the incomplete peptide is transported in an inactive form to the dendrite, bound to the stalled ribosome. Upon reactivation, the stalled ribosome completes protein synthesis, allowing for the newly-translated proteins to function at the synapse. Stalled polysomes can be detected using a cellular run-off assay where new polysome synthesis is blocked using an initiation inhibitor, actively translating polysomes are run off, and the remaining 'stalled' polysomes are detected using ribopuromycylation; this is a technique where puromycin is used to detect ribosomes with nascent polypeptides, and emetine is used to trap the puromycylated peptide on the ribosome (David et al., 2013). Using this assay we have found that most polysomes in dendrites of hippocampal cultures are stalled (Graber et al, 2013)
To examine whether L-LTP requires release of stalled polysomes, we will use chemically induced L-LTP in hippocampal cultures. As this paradigm is not well established, we are comparing activating L-LTP with (i) low Mg^2+-Glycine (Fortin et al., 2010), (ii) Forsklin (Shao et al., 2012), and the combination of Low-Mg^2+-Glycine and Forskolin. We are examining both the production of proteins known to be increased after L-LTP (CAMKII, S6 and PKM zeta) and the effect of L-LTP induction on the number of stalled polysomes using ribopuromycylation

(Tsokas et al., 2007; Eom et al., 2014; Ouyang et al., 1999).
Staufen1 (Stau1) is a protein which binds to double-stranded RNA and plays an important role in transporting mRNA to the dendrites (Vessey et al., 2008); down-regulation of Stau1 blocks L-LTP (Lebeat et al., 2008). As we have recently found that Stau2 is required for the regulation of stalled polysomes in mGLUR-LTD (Lebeau et al., 2011), it is possible that Stau1's requirement for L-LTP is also through the regulation of a distinct class of stalled polysomes. We have developed a lentivirus to knockdown Stau1 in hippocampal cultures and will report if it is required for the production of stalled polysomes and the increase in proteins during L-LTP.

Disclosures: K. Ginzberg: None. **M. Anadolu:** None. **T.E. Graber:** None. **W. Sossin:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

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Title: A combinatorial code of immediate-early genes encodes salient experiences

Authors: *A. CITRI;
The Hebrew Univ., Jerusalem, Israel

Abstract: How an individual responds to a situation is a function of cumulative past experience. We are interested in identifying plastic changes induced in the brain by experience, and understanding how they modify behavior in the future. We focus on experience-dependent plasticity at the molecular level, aiming to identify signatures of transcriptional dynamics that are unique to the encoding of aversive or rewarding experiences in the mesolimbic and limbic brain areas. Our entry point to this study is the comparative investigation of acute or repeated rewarding and aversiveexperiences. For each experience, we assessed the induction of immediate-early genes (0,1,2,4 hrs following the experience) in multiple brain regions: Nucleus Accumbens, Dorsal Striatum, Prefrontal Cortex, Amygdala, Lateral Hypothalamus,

Hippocampus and Ventral Tegmental Area. We investigated the transcriptional response to different experiences, by assessing gene expression patterns in naïve mice and mice exposed to acute or repeated experiences. Unique and robust transcriptional programs were identified in response to distinct experiences to the extent that each experience could be described by a pattern of transcription of a subset of genes in relevant structures. Furthermore, the recent experience of individual mice could be decoded with above 94% efficiency, solely based on the transcriptional induction of a subset of genes in a subset of brain structures. Dramatic differences were revealed in the encoding of aversive vs rewarding experiences. We observed a strong linkage between different features of an experience and the activation of sub-patterns of transcription in specific brain regions. Naturalistic rewarding experiences showed a development of the transcriptional response with repetition, while aversive experiences demonstrated a diminishment of the transcription imprint upon repetition. Our results reveal that different experiences are encoded by unique transcriptional patterns. Gene expression in distinct brain structures, dependent on the context and quality of the experience, represents encoding that may lead to different behavioral outcomes in the future. We propose the initiation of a new field of research, “Behavioral Transcriptomics”, aimed at utilizing transcription as an approach for investigation of mechanisms of experience-dependent circuit plasticity.

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Poster

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Title: Absence of the long non-coding rna bc1 disrupts spine morphology and experience-dependent plasticity and learning

Authors: *V. BRIZ[1], L. RESTIVO[2,3], E. PASCIUTO[1], K. JUCZEWSKI[4,5], V. MERCALDO[2], A. BORRECA[1,6], T. GIRARDI[7], R. LUCA[1,3], N. GUNKO[9], P. BAATSEN[9], R. POORTHUIS[10], H. MANSEVELDER[10], G. FISONE[5], M. AMMASSARI-TEULE[3], J. NYS[1], L. ATCKENS[8], P. KRIEGER[11], R. MEREDITH[10], C. BAGNI[1,12,6];
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Abstract: The brain cytoplasmic RNA BC1 is a long non-coding RNA known to be involved in neuronal translational control, partly via association with the Fragile X Mental Retardation Protein ribonucleoprotein complex. Absence of BC1 has been associated with altered glutamatergic transmission and impaired adaptive behavior. Here, we investigated the role of BC1 RNA in the primary somatosensory cortex, a well-defined system to explore the link between dendritic spine morphology and synaptic connectivity. We found that spine density and spine head size are abnormal in barrel cortex principal neurons of BC1 knock out (KO) mice. Consistent with these results, BC1 KO mice have larger excitatory postsynaptic currents and spontaneous activity than their wild type littermates in this cortical region, as evidenced by *ex vivo* and *in vivo* electrophysiology. In addition, neurons lacking BC1 RNA show increased basal protein translation and higher postsynaptic levels of several glutamate receptor subunits and associated structural proteins. Finally, we provide evidence that barrel cortex structural plasticity in response to whisker deprivation and novel object recognition are impaired in BC1 KO mice. These findings highlight the importance of this non-coding RNA in synaptic structure and function as well as experience-dependent plasticity and learning. Furthermore, our study provides a novel animal model to understand how spine dysmorphogenesis leads to alterations in synaptic transmission and behavior, which could be particularly relevant in the context of intellectual disabilities.

Disclosures: **V. Briz:** None. **L. Restivo:** None. **E. Pasciuto:** None. **K. Juczewski:** None. **V. Mercaldo:** None. **A. Borreca:** None. **T. Girardi:** None. **R. Luca:** None. **N. Gunko:** None. **P. Baatsen:** None. **R. Poorthuis:** None. **H. Mansevelder:** None. **G. Fisone:** None. **M. Ammassari-Teule:** None. **J. Nys:** None. **L. Atckens:** None. **P. Krieger:** None. **R. Meredith:** None. **C. Bagni:** None.

Poster

403. Transcription and Translation: Synaptic and Circuit Plasticity

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 403.17/H4

Topic: B.08. Synaptic Plasticity

Support: Polish National Science Center grant (SONATA BIS 2) DEC-2012/07/E/NZ3/01814.

Title: Serum response factor regulates structural plasticity of dendritic spines during development.

Authors: *K. KALITA-BYKOWSKA, A. KRYSIAK, A. SUSKA, S. LESKI, L. KACZMAREK;
Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Re-arrangement of neuronal networks plays an important role in proper circuitry formation. Dendritic spines' shape changes from thin elongated filopodia-like structures to stable mushroom dendritic spines during brain development. Serum Response Factor (SRF), one of the major transcription factors in the brain, plays a prominent role in regulating various programs of gene expression in the adult brain in response to stimulation. The aim of our study was to investigate potential novel SRF functions in the regulation of structural plasticity during brain development. We found that SRF protein expression change within the course of postnatal development of mouse hippocampus *in vivo*. SRF knockdown in hippocampal primary cultures resulted in increased number of filopodia-like protrusions and decreased number of mushroom spines with the general lack of changes in the overall density of dendritic spines. Moreover, spines of SRF knockdown neurons exhibited altered morphology, highlighted by increased length and area of filopodia-like and long spines. Furthermore, SRF-depleted neurons had lower level of surface AMPAR GluR1 subunit. We showed that the number of functional synapses and their activity was lower in SRF-depleted cells as shown by a reduction in the frequency and amplitude of miniature excitatory postsynaptic currents. These findings indicate that SRF regulates transcription of genes essential for synapse/spine formation and maturation during hippocampal development.

Disclosures: K. Kalita-Bykowska: None. **A. Krysiak:** None. **A. Suska:** None. **S. Leski:** None. **L. Kaczmarek:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 404.01/H5

Topic: B.09. Intrinsic Membrane Properties

Support: SNF PP00P3_128415

Title: Network dynamics of nociceptive processing in the anterior cingulate cortex

Authors: F. KASANETZ, M. SANTELLO, *T. NEVIAN;
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Abstract: The Anterior Cingulate Cortex (ACC) plays a central role in the interpretation and evaluation of the affective and emotional components of pain. Accumulating evidence indicates that abnormal neuronal plasticity and a resulting hyperactivity of the ACC is the cause for the manifestation of the emotional distress that characterizes chronic pain conditions. However, little is known on how the functional organization of ACC microcircuits is affected in chronic pain. Apart from its involvement in pain perception, the ACC is engaged in a variety of other cognitive and emotional processes such as working memory, inhibitory control, conflict monitoring, fear, attention, salience and reward expectancy. How neuronal populations in the ACC can be involved in such a diversity of functions is a matter of debate. One intriguing hypothesis would be that the ACC is composed of multiple sub-circuits mediating separate aspects of behavior. Here we addressed whether the ACC possesses specialized neurons that process nociceptive information and how this putative microcircuit organization is affected in chronic neuropathic pain. We also focused on the role and activity of dendritic sub-compartments of pyramidal neurons in information processing in the ACC. Using in vivo recording of spiking activity in the mouse ACC, we have identified a subpopulation of neurons that are activated in response to nociceptive stimulation. Interestingly, this “nociceptive neurons” showed a preferential increase in spontaneous activity during chronic pain, suggesting that ACC hyperactivity might be restricted to a sub-network of pain-related cells. In order to gain insight into the organization and plasticity of the ACC, we will present data combining in vivo two-photon calcium imaging of neuronal populations and dendritic sub-compartments with neuronal tracing and synaptic silencing techniques to uncover the functional properties and neuronal identity of ACC “nociceptive neurons” and its modulation by chronic pain.

Disclosures: **F. Kasanetz:** None. **M. Santello:** None. **T. Nevian:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 404.02/H6

Topic: B.09. Intrinsic Membrane Properties

Support: OIST Graduate University

Title: Exploring input-output relations of neurons *In vivo*

Authors: C. J. ROOME, *B. KUHN;
OIST Grad. Univ., Onna-Son, Okinawa, Japan

Abstract: Measuring input-output relations of single neurons in vivo is very important for understanding how the brain works. So far, the most complete input-output relations were measured in brain slices, which lack physiological input to dendrites and soma. Here we combine two-photon microscopy and electrophysiology to simultaneously measure dendritic voltage and calcium signals (inputs) and somatic output from Purkinje cells (PC) in vivo. To record dendritic voltage optically we labelled single PCs with the voltage sensitive dye ANNINE-6plus, using a chronic cranial window with access port (Roome and Kuhn, 2014). For dendritic calcium recording, adeno-associated viruses delivering the gene of the genetically encoded calcium indicator GCaMP6f, was injected prior to ANNINE-6plus labelling. Extracellular electrophysiology was also performed at the labelled PC soma to record their somatic activity. Combing techniques allows measurement of voltage and calcium changes in the PC dendrite and simultaneous electrical recording from the PC soma in the cerebellum of awake mice. By dissecting input-output relations at a single cell level within the intact brain, we aim to address important questions concerning neuronal computations in vivo.

Disclosures: C.J. Roome: None. **B. Kuhn:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

Location: Halls B-H

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Program#/Poster#: 404.03/H7

Topic: B.09. Intrinsic Membrane Properties

Support: MH109091

NS085729

MBL Fellowship

Title: Glutamate-mediated plateau potentials studied by simultaneous multi-site dendritic sodium and calcium imaging

Authors: *S. D. ANTIC[1], K. MIYAZAKI[2], W. N. ROSS[2];
[1]Neurosci, UConn Hlth., Farmington, CT; [2]Physiol., New York Med. Col., Valhalla, NY

Abstract: Strong synaptic inputs generate glutamate-mediated plateau potentials in basal dendrites of cortical pyramidal neurons (Milojkovic *et al.*, 2004). Plateau potentials propagate into the soma, sometimes causing relatively large amplitude (~20 mV) sustained (~200 ms) somatic depolarizations, often accompanied by bursts of action potentials. Such dendritic plateau potentials are regenerative in nature and dependent on mixtures of activated conductances (AMPA, NMDA, Na^+ channels, voltage-gated Ca^{2+} channels (VGCC) and K^+ channels) in a process dubbed "spike-chain mechanism" (Schiller *et al.*, 2000). Because activation of one conductance can influence activation of other conductances through voltage and/or resulting ion fluxes (e.g. [Ca2+]i), it is difficult to determine the precise relative contribution and order of spikes in the dendritic spike-chain event based solely on the actions of selective channel antagonists on the spike waveform. One way to determine more information about the relevant dendritic currents is to observe concentration changes resulting from fluxes of ions to which receptors and channels are permeable. For example the influx of Na^+ reports activity of dendritic AMPA, NMDA and Na^+ channels, while the influx of Ca^{2+} reports activity of NMDA and VGCCs. In the present study we simultaneously monitored dendritic Na^+ and Ca^{2+} influxes during subthreshold and suprathreshold glutamate-mediated potentials. Simultaneous Na-Ca imaging (Miyazaki & Ross, 2015) was used to detect concentration changes in L5 pyramidal neurons in brain slices cut from (male and female) rat medial prefrontal cortex. [Na+]i changes were detected with either SBFI or ANG-2; [Ca2+]i changes were detected with either OGB-5N, OGB-1 or Bis-fura-2. Focal glutamate microiontophoresis was used to trigger plateau potentials. We observed Ca^{2+} transients from multiple locations along dendrites. The largest responses were at the input site and were blocked by APV. Application of VGCC antagonists reduced the Ca^{2+} signal beyond the input site. Na^+ transients were detected at the input site only. They persisted in the presence of TTX showing that they were receptor mediated. In many experiments most of the Na^+ signal remained after the addition of APV suggesting a strong AMPA receptor component in these responses.

Disclosures: **S.D. Antic:** None. **K. Miyazaki:** None. **W.N. Ross:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 404.04/H8

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant MH104602

Title: Somatic membrane potential modulates the propagation of dendritic spikes in CA1 pyramidal neurons

Authors: *T. BOCK, S. A. SIEGELBAUM;
Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Active signal propagation in the apical dendrites of pyramidal neurons (PNs) can amplify synaptic potentials, modulate action potential (AP) firing and contribute to synaptic plasticity. Dendritic calcium spikes have been thoroughly investigated in layer 5 PNs in acute cortical slice preparations, where they readily propagate to the soma and help induce burst firing of APs. Although in vivo recordings from hippocampal CA1 PNs suggest that dendritic spikes can trigger burst firing in the soma, recordings in acute slices indicate that these spikes propagate poorly to the soma, and so have only a limited impact on somatic output. As the in vivo resting potential is significantly depolarized relative to ex vivo conditions, we investigated the hypothesis that the resting potential can influence the efficacy of dendritic spike propagation. We performed simultaneous dual whole cell recordings from the soma and the distal apical dendrites of CA1 PNs in acute hippocampal slices. The propagation of dendritic spikes triggered by current injection or synaptic stimulation was strongly modulated by the somatic membrane potential. When the resting potential of the soma was depolarized, the efficacy of propagation was markedly increased. Depolarizing the resting membrane potential from its normal ex vivo value of -70 mV to its in vivo value of -55 mV increased the somatic depolarization produced by a dendritic spike by 398 % (± 69 %), which can now induce somatic burst firing. This effect is likely mediated by the inactivation of dendritic A-type potassium channels, as the application of 5 mM 4-AP enhanced the propagation of dendritic spikes at the normal negative resting potential. Furthermore, this modulation of dendritic spike propagation has a profound impact on plasticity mechanisms that require dendritic spikes. Input time dependent plasticity (ITDP) is induced by the precise pairing of entorhinal cortex inputs to distal dendrites with Schaffer collateral inputs to proximal dendrites. Normally, ITDP requires 90 paired stimuli at 1 Hz, which leads to the firing of dendritic spikes. However, somatic depolarization facilitates the propagation of dendritic spikes during synaptic pairing and also facilitates the induction of ITDP to <40 paired events. These experiments show the importance of somatic resting membrane potential for the electrical coupling of subcellular compartments, such as the distal apical

dendrite and the soma. As CA1 PNs are much more depolarized *in vivo*, due to constant low-level synaptic input, than in acute slices, we hypothesize that dendritic spikes have a much larger impact on plasticity mechanisms and on AP firing, under physiological conditions.

Disclosures: T. Bock: None. **S.A. Siegelbaum:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

Location: Halls B-H

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Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant NS085729

Title: Synaptically activated sodium changes in dendritic spines of rat hippocampal pyramidal neurons

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

Abstract: Measurements of physiologically activated $[Na^+]_i$ changes can reveal information about functions that $[Ca^{2+}]_i$ changes are not directly sensitive to. One example is synaptic activation of AMPA receptors, which are permeable to Na^+ but not to Ca^{2+} in many neurons. A second example is activation of voltage gated Na+ channels (VGSCs), which usually are detected optically by monitoring $[Ca^{2+}]_i$ changes caused by the associated voltage changes. To examine these kinds of events in hippocampal slices we injected individual pyramidal neurons with pairs of Na^+ and Ca^{2+} indicators and measured fluorescence from both indicators simultaneously at high speed (250 Hz in each channel) with a CCD camera (Miyazaki and Ross, 2015). Usually we measured $[Na^+]_i$ changes in one channel and $[Ca^{2+}]_i$ changes in the other channel. Sometimes, to enhance the $[Na^+]_i$ signal, we used two different Na^+ indicators that generated fluorescence changes of opposite sign in response to a $[Na^+]_i$ increase. This procedure enhanced the S/N of the response and eliminated common mode noise from movements. Synaptic shocks in the Str. radiatum at low intensity evoked epsps without spikes. These responses were sometimes associated with both $[Na^+]_i$ and $[Ca^{2+}]_i$ changes that were localized to a few microns on the dendrite. To enhance the success rate we used paired stimuli at 5 ms intervals. This improvement was probably due to facilitation since greater shock separation clarified that the signal came from the second stimulation. APV+CPP blocked most of the localized $[Ca^{2+}]_i$ increase but had only a small effect on the $[Na^+]_i$ increase, indicating that entry through NMDA receptors was responsible for most of the $[Ca^{2+}]_i$ increase, while entry through

AMPA receptors was responsible for most of the $[Na^+]_i$ increase. Including QX-314 in the pipette did not block the $[Na^+]_i$ increase showing that little signal was due to entry through VGSCs. This result was consistent with the finding that backpropagating spikes alone evoked only small $[Na^+]_i$ increases in the dendrites. Since other experiments have established that synaptically activated AMPA receptors are located exclusively on spine heads we conclude that the localized $[Na^+]_i$ increases in the presence of APV+CPP results from Na^+ entry into spines. In many cells the rise time of the $[Na^+]_i$ increase at the center of the response was less than 10 ms, while the rise time at locations several microns away was slower, suggesting Na^+ entry in the spine and diffusion to sites on the dendrites. The decay time at many spines was 50 ms or less. Using a simple model for diffusion (Svoboda et al., 1996) this fast recovery suggests a low neck resistance in these spines.

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

Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.09. Intrinsic Membrane Properties

Support: NS044163 (NINDS)

VA Merit Review 5 I01 BX000386

VA Epilepsy Center of Excellence

Title: Dendritic Integration in the basal and proximal apical oblique dendrites of two distinct layer 5 pyramidal neuron populations

Authors: *N. C. DEMBROW[1,2], G. S. NEWKIRK[3], W. SPAIN[3,2];
[1]Univ. of Washington Dept. of Physiol. and Biophysics, Seattle, WA; [2]VA Epilepsy Ctr. for Excellence, Seattle, WA; [3]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: The basal and apical oblique dendrites receive a significant proportion of the synaptic input to neocortical layer 5 (L5) pyramidal neurons. When measured somatically, each individual input is quite small. However, when several synaptic inputs onto a single dendritic branch are clustered in time, they combine to trigger a much larger amplitude, supralinear response. Such nonlinear integration is critical for information processing and signal propagation within the cortical circuit. We compared branch integration in the basal dendrites and the proximal apical obliques for two different L5 pyramidal neuron populations in the motor cortex using the BAC

mouse lines: Etv1-egfp (etv1) and Thy1-yfp-h (thy1). Spines were activated by photolyzing MNI-glutamate with brief focused pulses of light (200 us at 720 nm). Individual spine responses were calibrated to match previously established equivalent amplitude and rise times of sucrose-evoked responses for each neuron type. To characterize branch integration, we selected multiple (~30) spines evenly distributed along a single dendritic branch. For both thy1 (n = 17) and etv1 (n = 13), near-synchronous activation triggered supralinear responses of equivalent amplitudes and rise times. Furthermore, thy1 and etv1 dendrites did not require a significantly different minimum number of spines to trigger a supralinear event. However, the half-widths and thus the area of these responses were significantly greater in the etv1 neurons (events in etv1 neurons were 61% wider, 77% greater in area). These data suggest that recruitment of active events in the thin dendrites of etv1 neurons may exert longer-lasting and thus a more profound effect upon action potential firing. We next tested how much temporal clustering was necessary to produce supralinear events. For all dendritic compartments in both neuron types some temporal clustering was necessary: spine stimulation separated by > 5 ms (200 Hz), produced a voltage response identical to the linear sum. But the sensitivity to temporal clustering differed depending upon the neuron type and dendritic compartment. Thy1 basal dendrites exhibited a threshold-like sensitivity to temporal clustering. In contrast, the amplitude of supralinear responses in etv1 basal dendrites and thy1 proximal apical obliques increased with stimulation frequency in a graded manner. Thus the input sensitivity and the output response both depend upon the dendritic compartment and the neuron type in question. As such, information processing by these different units may be expected to be distinct.

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

Poster

404. Synaptic Excitability and Dendritic Integration

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Program#/Poster#: 404.07/H11

Topic: B.09. Intrinsic Membrane Properties

Support: Swiss National Science Foundation (grant 31003A_162558)

Title: Dendritic NMDA spikes: from full-blown to graded boosting supralinearities. What causes the switch?

Authors: ***F. BRANDALISE**, U. GERBER;
Brain Res. Inst., Univ. of Zurich, Zuerich, Switzerland

Abstract: The recent demonstration that dendritic spikes are also generated *in vivo* (Gambino et al., 2014; Grienberger et al., 2014; Palmer et al., 2014; Bittner et al., 2015; Sheffield & Dombeck, 2015) has led to renewed investigation of their roles in dendritic computation for LTP induction (Chicon & Gan, 2015; Kim et al., 2015) and neuronal up-down state (Smith et al., 2013; Oikonomou et al., 2014). Among the various types of dendritic events, NMDA spikes are of particular interest because of their association with input timing-dependent synaptic plasticity (ITDP). Interestingly, an examination of input-output data shows that NMDA spikes can present either as full-blown spikes with a distinct threshold (Schiller et al., 2000; Major et al., 2013) or as events with graded amplitudes (Branco & Hausser, 2011). At present, the mechanisms underlying this difference remain unclear. We addressed this question at CA3 recurrent synapses where we induced associative LTP by repetitive pairing of a CA3-CA3 input followed by a mossy fiber input (Brandalise & Gerber, 2014). The amount of the potentiation decreased as a function of the distance between the mossy fiber and the CA3 recurrent inputs. We then repeated this protocol, but after inducing LTP at the mossy fiber input. In this case, we observed that at distal CA3 recurrent synapses the probability of evoking an NMDA spike was increased, as was the amount of LTP. At proximal synapses, however, although the number of NMDA spikes also increased, additional potentiation did not occur. Further analysis revealed that the NMDA spikes at non-potentiated synapses manifested as full-blown events, whereas NMDA spikes at already potentiated synapses turned into graded amplitude events. We then tested the hypothesis that a change in the synaptic AMPA/NMDA receptor ratio is responsible for the difference in the NMDA spike response. We therefore induced LTP at CA3 recurrent synapses employing a standard spike timing-dependent LTP protocol (STDP), which is known to increase the AMPA/NMDA receptor ratio (Debanne et al., 1998). We found that an increase in this ratio favored the generation of graded NMDA spikes. Subsequent application of an STDP LTD protocol converted the graded dendritic supralinearity back to full-blown NMDA spikes. These findings indicate that the initial state of potentiation of a synapse is a key factor determining whether NMDA spikes are induced as graded or as full-blown responses. This phenomenon may represent a homeostatic mechanism that serves to curtail excessive potentiation of synapses. Thus, NMDA spikes can act not only as causal triggers of synaptic plasticity but also as modulators of neuronal up-down state.

Disclosures: F. Brandalise: None. **U. Gerber:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.09. Intrinsic Membrane Properties

Support: Boehringer Ingelheim Fonds

Wellcome Trust WT094077MA

ERC AdG 250345

Title: Dendritic spikes determine input selectivity in pyramidal cells

Authors: L. GOETZ, M. R. GROEN, *A. ROTH, M. HAUSSER;
Univ. Col. London, London, United Kingdom

Abstract: Neurons compute by nonlinearly transforming spatiotemporal patterns of synaptic input into action potential (AP) output. Features of this input-output transformation during sensory processing in visual cortex (V1) include bursts of spikes riding on dendritic plateau potentials (Smith et al., Nature 2013). However, it is unclear which spatiotemporal patterns of synaptic inputs generate these local spikes and underlying plateaus, and how they in turn contribute to the initiation of APs. To address this issue quantitatively, we constructed a model of the presynaptic input received by a layer 2/3 pyramidal neuron during visual stimulation with drifting gratings, using experimental data on the statistics of spontaneous and sensory-evoked APs in neurons of mouse V1 and their synaptic connectivity. We make the simplest possible (and conservative) assumption that synaptic inputs are independent of each other, and randomly distributed in space over the postsynaptic dendrites as well as randomly activated in time. We use our presynaptic input model to drive a detailed active compartmental model of a postsynaptic layer 2/3 pyramidal neuron that satisfies an extensive set of constraints from experiments *in vitro* and *in vivo*.

This model successfully recapitulates the *in vivo* experimental data, generating bursts of fast local dendritic spikes, which are associated with dendritic plateau potentials. Both fast spikes and plateau potentials are heterogeneous in amplitude and time course, and depend primarily on sodium channel and NMDA receptor activation, respectively. We measured the distribution of the number of synaptic inputs required to trigger a fast local dendritic spike or a plateau potential, and how this number depends on the synaptic weights of the synapses involved. We find that the input patterns triggering dendritic regenerative events are sparse, and in some cases a single strong input synapse can trigger fast local spikes. Next, we established global conditions under which fast local spikes and NMDA plateaus influence neuronal output tuning. Specifically, we demonstrate the causal effect of fast local spikes and plateau potentials on orientation selectivity of AP output by selectively switching off the voltage dependence of the underlying conductances.

To conclude, we provide a quantitative prediction of numbers, strengths and spatiotemporal patterns of synaptic inputs generating dendritic spikes *in vivo*. Our model mechanistically explains how differently tuned synaptic inputs, which are distributed randomly over the dendritic tree, can give rise to orientation-tuned dendritic spikes, and in turn to the stimulus selectivity of axonal output.

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

Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.09. Intrinsic Membrane Properties

Support: APP1082257

DP130101630

Title: Rostro-caudal gradient of the dendritic integrative properties of layer 5 pyramidal neurons across the primary visual cortex

Authors: L. N. FLETCHER, *S. R. WILLIAMS;
Queensland Brain Inst., Brisbane, Australia

Abstract: The thickness of the neocortex varies over the neocortical mantle, a property that determines the size of the dendritic arbor of pyramidal neurons. In order to preserve canonical cell-class properties across the neocortex, the electrical architecture of the dendritic arbor must parallel such morphological changes. The conservation of the integrative properties of a defined class of neuron with dendritic size has, however, not been directly explored. Here we use high-resolution anatomical reconstruction, multi-site somato-dendritic electrophysiological recordings and computational modelling approaches to demonstrate that the physical size, electrotonic architecture, and mode of dendritic integration of layer 5B pyramidal neurons vary as a gradient across the rostro-caudal axis of the Wistar rat primary visual cortex. Our findings reveal that the integrative capacity of layer 5B pyramidal neurons transforms from multi-compartment, layered computations, to compact axo-somatic integration across the primary visual cortex. These data challenge the view that neocortical neuronal populations carry out canonical computations.

Disclosures: L.N. Fletcher: None. **S.R. Williams:** None.

Poster

404. Synaptic Excitability and Dendritic Integration

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Program#/Poster#: 404.10/H14

Topic: B.09. Intrinsic Membrane Properties

Title: Three-dimensional calcium imaging of mouse hippocampal neuronal ensembles during Sharp wave-ripple complexes

Authors: *D. PALFI[1], B. CHIOVINI[2], L. JUDAK[2], G. SZALAY[2], G. JUHÁSZ[1], G. KATONA[1], B. ROZSA[2];
[1]Peter Pazmany Catholic Univ., Budapest, Hungary; [2]IEM HAS, Budapest, Hungary

Abstract: Sharp wave-ripple (SPW-R) complexes is driven by an interaction between hippocampal excitatory pyramidal cells and inhibitory neurons. A SPW event is the most synchronous rhythm of the hippocampus where only five to ten percent of the pyramidal cell population is activated. During network activity synaptically activated dendritic segments can be participate in the neuronal engram. In our previous studies demonstrated the existence of dendritic calcium spikes in fast spiking parvalbumin interneurons (FS-PV INs) during sharp wave-ripple (SPW-R) activities. Until now, the dendritic integration mechanisms of the hippocampal pyramidal neurons (PYR) during SPW-R remain elusive. In this work we present multiple 3D, acousto-optical, two-photon laser-scanning technologies to monitor neuronal activity at different scales with genetically-encoded calcium indicators in a near-cubic-millimeter scan range (up to 700 × 700 × 1,400 μm3), with a high scanning speed (up to 1 Mhz), with high (<500 nm) resolution in the center core, and less than 1.9 x 1.9 x 7.9 μm3 resolution throughout the whole scanning volume. We used volumetric random-access calcium imaging of spontaneous activity from hundreds of neurons. We applied 3D trajectory scanning technique to measure multiple dendritic segments in vivo with motion compensation possibility. Finally we introduced an expansion of trajectory scanning technique to monitor the activity from more than 100 cells in vivo while obtaining information for motion correction.

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Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant U01EB017695

Title: Dendritic morphology of corticospinal and crossed-corticostriatal neurons in mouse primary motor cortex

Authors: *B. A. SUTER[1], S. A. NEYMOTIN[2,4], G. M. G. SHEPHERD[5], W. W. LYTTON[3,6]; [1]IST Austria / Jonas Group, Klosterneuburg, Austria; [2]Physiol. & Pharmacol., SUNY Downstate, Brooklyn, NY; [3]Physiol. & Pharmacol., SUNY Downstate, Brooklin, NY; [4]Neurosci., Yale University, Sch. of Med., New Haven, CT; [5]Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [6]Kings County Hosp. Ctr., Brooklyn, NY

Abstract: In neocortex, layer 5 is home to at least two types of pyramidal neurons, which can be distinguished by their projection targets - with roughly half projecting to subcerebral targets (pyramidal-tract, PT), including the spinal cord, and the rest extending axons only within the ipsi- and contra-lateral telencephalon (intratelencephalic, IT). These classes differ in intrinsic electrophysiology as well as local and long-range synaptic connectivity. They also differ in dendritic morphology, with PT neurons forming a “thick tuft” in layer 1, while IT neurons have “thin” tufts, or none at all. In rat frontal cortex, a quantitative analysis found additional characteristic differences between classes, and a sublaminar differentiation within ITs (Morishima & Kawaguchi, 2006). In mouse motor cortex, the quantitative dendritic differences between these classes have not been fully characterized, especially in the non-tuft regions. Previously, we found that PT dendrites (N=24 corticospinal neurons) are very uniform, with a constant fraction (~25%) in the tuft, independent of soma depth within layer 5B. Notably, about 65% of PT dendrites are located peri-somatically, where synapses are electrotonically closer to the soma. We thus wished to quantitatively compare the dendritic length-density across PT and IT populations, to ascertain (a) the relative density of peri-somatic dendrites, and (b) whether IT-type dendrites form a homogenous pattern, as is the case for the PT population.
We generated three-dimensional dendritic reconstructions of retrogradely-labeled ITs (N=17 crossed corticostriatal neurons) and analyzed them for length-density and branching patterns. We find that PTs have more dendrite than ITs in all compartments. PT dendrites are ~2x longer, with the most dramatic difference in the tuft (7.5x). In ITs the tuft length decreases as a function of soma depth - the deepest ITs lacking any tuft. Peri-somatic length-density is 1.5x greater in PT than in IT, and in part because PTs form a greater number of basal root branches.
Together, these findings demonstrate a combination of projection-specific and sublaminar dendritic patterning, leading to significant differences in the cortical volume sampled by individual pyramidal neurons, and the density with which this space is sampled, by both the apical tuft and peri-somatic dendritic compartments.
We used whole-cell somatic current-clamp recordings of the reconstructed neurons to generate NEURON models for both cell types via evolutionary optimization algorithms. We plan to use these models to investigate the contribution of dendritic morphology to the diversity of firing responses observed in layer 5.

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Poster

404. Synaptic Excitability and Dendritic Integration

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Title: Schizophrenia as a disorder of cellular excitability: Insights from computational models of cortical neurons and cardiac pacemaker cells

Authors: *T. MÄKI-MARTTUNEN[1], G. HALNES[3], A. DEVOR[4], G. T. LINES[5], A. G. EDWARDS[5], A. TVEITO[5], A. WITOELAR[2], F. BETTELLA[2], S. DJUROVIC[2], Y. WANG[2], A. M. DALE[4], G. T. EINEVOLL[3], O. A. ANDREASSEN[2];
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Abstract: Schizophrenia (SCZ) is a highly heritable mental disorder with a high burden of morbidity and large social impacts. Proposed disease mechanisms range from altered brain structure to deficits in synaptic transmission and immune system functions. One of the generic hypotheses addresses SCZ as a disorder of cortical excitability (O'Donnell 2008, *Cortical Deficits In Schizophrenia*, Springer). Altered membrane excitability as a disease mechanism is supported by a recent genome-wide association study that revealed 108 SCZ-associated genetic loci, many of which implicate genes encoding ion channels and Ca^{2+} transporters (Ripke et al. 2014, *Nat Gen* 45.10).
Although SCZ symptoms are primarily related to brain and cognition, SCZ patients also have an elevated risk of cardiac dysfunction. Part of the risk is inflicted by long-term use of antipsychotics, while part could be of genetic origin (Andreassen et al. 2013, *Am J Hum Genet* 92.2; Fujii et al. 2014, *PLoS ONE* 9.6: e98555). The elevated risk manifests in increased mortality rates among the patients, which prevail even when corrected for the increased numbers of deaths caused by accidents and suicide (Brown 1997, *Br J Psych* 171.6).
In this work, we employ biophysically detailed computational models of cortical neurons and cardiac pacemaker cells to study the contribution of SCZ and cardiac disease-associated genes to cellular excitability and functioning of these cells. We focus our analyses on two central cell

types: layer V pyramidal cells (L5PCs) in the cortex and sinoatrial node cells (SANCs) in the myocardium. The apical tuft of an L5PC is considered a biological substrate for cortical associations providing high-level context for low-level (e.g., sensory) inputs that arrive at the perisomatic compartment, and the ability of L5PC to integrate these inputs has been proposed as one of the mechanisms that could be impaired in hallucinating patients (Larkum 2013, *Trends Neurosci* 36.3). The SANCs, in turn, have a key role in controlling heart rate as the primary pacemakers of the mammalian heart. We show that subtle variants of SCZ-associated genes (modeled as in Mäki-Marttunen et al. 2016, *Biol Psych: Cogn Neurosci Neuroim* 1, 49-59) cause alterations in the L5PC ability to integrate apical and perisomatic inputs. Moreover, we show that these genetic variants affect electrical excitability of both L5PCs and SANCs, but often in opposite directions. Our results provide a plausible mechanistic explanation for the shared genetic etiology between SCZ and cardiac disease. In the future, this type of modeling approach may contribute in the development of the next generation antipsychotics that are free from cardiac side effects.

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Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.07. Synaptic Transmission

Support: Regione Autonoma della Sardegna, L.R. 7/2007-CRP10810/2012

Title: Down-regulation of TrkB expression and signaling by the anti-convulsant drug valproic acid.

Authors: *S. DEDONI, M. C. OLIANAS, P. ONALI;
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Abstract: Several studies have shown that the anti-convulsant and mood-stabilizer drug valproic acid (VPA) can regulate the levels of brain-derived neurotrophic factor (BDNF) both in brain and cultured neural cells, but relatively little is known on whether VPA can affect the neuronal responses to BDNF. In the present study we investigated the effects of VPA on the expression and signaling of the BDNF receptor TrkB in retinoic acid-differentiated SH-SY5Y human

neuroblastoma cells and primary cultures of mouse cortical neurons. We found that prolonged exposure (24 h) of SH-SY5Y cells to VPA (1 mM) inhibited BDNF-induced phosphorylation of different signaling molecules regulated by TrkB, including Akt, GSK-3β and PLCγ1. The VPA inhibitory effects were associated with down-regulation of TrkB expression and inhibition of BDNF-induced transphosphorylation of TrkB. Similar results were obtained in mouse primary cortical neurons, where VPA (1 mM) reduced the TrkB protein levels and inhibited BDNF-induced Akt activation. The effects of VPA appeared to be specific for TrkB, as in SH-SY5Y cells the drug failed to affect TrkA levels and enhanced the expression of the common neurotrophin receptor p75-NTR. In agreement with its ability to inhibit histone deacetylases (HDACs), VPA markedly increased the acetylation of histone H2B at Lys5. Interestingly, the VPA inhibitory effect on BDNF signaling and TrkB expression was mimicked by other HDAC inhibitors, such as sodium butyrate, trichostatin A and MS-275, but not valpromide, a VPA derivative which did not enhance histone acetylation, indicating the implication of epigenetic mechanisms. As BDNF overactivity may play a role in the patogenesis of temporal lobe epilepsy and mania, the present study suggests that down-regulation of TrkB may contribute to the anticonvulsant and mood-stabilizing actions of VPA.

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Poster

404. Synaptic Excitability and Dendritic Integration

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Topic: B.07. Synaptic Transmission

Support: HHMI

Title: Inhibition enhances rate and phase coding of place in hippocampal CA1

Authors: ***C. GRIENBERGER**, A. D. MILSTEIN, K. C. BITTNER, S. ROMANI, J. C. MAGEE;
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Abstract: Recent work suggests that all CA1 principal neurons receive a broad range of spatially tuned synaptic input that encodes for all relevant features of an environment. This arrangement is advantageous in that it provides a high degree of flexibility for place cells to acquire or add new place fields through rapid input strength changes. However, it can also be a cause of noise, potentially degrading the accuracy of the hippocampal place code. How, then, does a place cell maintain selectivity for particular locations? Here, we examined the ability of inhibitory input to

reduce the impact of broadly tuned excitatory input on the spatially tuned activation of place cells by performing whole-cell recordings of CA1 pyramidal neurons and interneurons on head-fixed mice running on a linear track treadmill. Using this approach, we found that unlike pyramidal neurons, hippocampal interneurons lack spatial modulation of their subthreshold membrane potential, which is also reflected in the lack of place specific action potential firing. Next, using optogenetic hyperpolarization of these interneurons (by Arch expression in VGAT-ires-cre mice and Gad2-ires-cre mice), we found that a constant level of broadly tuned synaptic inhibition (1) enhances the spatial tuning of CA1 place cells by selectively countering out-of-field inputs, (2) helps maintain the place field location by controlling the occurrence of dendritic plateau potentials and (3) reduces the impact of out-of-field inputs on the phase of the intracellular oscillations that drive theta phase precession in CA1 place cells. Finally, we turned to a biophysically detailed simulation of the hippocampal microcircuit incorporating our experimental findings to investigate the mechanisms underlying the effect of inhibition. Taken together, our results demonstrate that inhibition greatly contributes to the sparse structure of the hippocampal network activity by controlling for the relatively broad tuning of spatial inputs that the CA1 microcircuit receives from its afferent brain areas.

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Poster

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ANR-2010-BLANC-1411

Title: Synaptic input distribution plays a role in the dendritic computation of motion direction in the retina

Authors: *A. VLASITS[1], R. D. MORRIE[2], A. TRAN-VAN-MINH[4,5], A. BLECKERT[6], C. F. GAINER[3], V. DUTELL[3], D. A. DIGREGORIO[4,5], M. B. FELLER[1,2];
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Abstract: Starburst amacrine cells are a critical component of the direction selective circuit in the retina. They are axon-less inhibitory interneurons, and therefore starburst amacrine cell dendrites contain both input and output sites. These dendrites present an opportunity to examine the precise role of functional sensory input location on dendritic computations. Using visual receptive field mapping, glutamate uncaging, two-photon Ca^{2+} imaging, and genetic labeling of putative synapses, we identified a unique arrangement of excitatory inputs and inhibitory release sites on starburst amacrine cell dendrites: excitatory inputs are skewed away from release sites. By comparing computational simulations with Ca^{2+} transients recorded near release sites, we show that this anatomical arrangement of inputs and outputs supports a dendritic mechanism for computing motion direction. Direction-selective Ca^{2+} transients persist when lateral inhibition is blocked by a GABA-a receptor antagonist, though directional tuning is reduced. These results indicate a synergistic interaction between dendritic and circuit mechanisms for generating direction selectivity. We explore the role of the dendrite-intrinsic and circuit-level components in the velocity-tuning of release sites. Our study highlights the important role of functional input location in the computations neurons are able to perform.

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Poster

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Topic: B.07. Synaptic Transmission

Title: Role of synaptic amplification in spatial selectivity in a biophysical model of the CA1 microcircuit

Authors: *A. D. MILSTEIN, C. GRIENBERGER, J. C. MAGEE, S. ROMANI;
Hhmi/Janelia Farm, Ashburn, VA

Abstract: Recent work suggests that CA1 place cells receive spatially tuned synaptic input corresponding to a broad range of locations in physical space, endowing place cells the flexibility to acquire spatially selective receptive fields in many locations through the plastic tuning of synaptic weights. However, this predicts a high degree of spatial and temporal synchrony in the pattern of presynaptic activity that a place cell receives, even at locations outside of its spatial receptive field, which is expected to engage presynaptic facilitation and postsynaptic amplification mechanisms. How, then, does a place cell maintain selectivity for locations with higher synaptic weights or increased input density? Here we present a biophysically detailed model demonstrating a role for broadly tuned synaptic inhibition in sharpening the spatial selectivity of CA1 place cells by suppressing membrane fluctuations driven by voltage-dependent amplification in spines and dendrites, consistent with experimental data from in vivo intracellular recordings. We also compare and contrast the features of place fields constructed by three alternative mechanisms: biased synaptic weights, biased input density, and spatial clustering of temporally correlated inputs on dendritic branches.

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Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

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Title: Inactivation of the hippocampo-septal GABAergic feedback modulates theta rhythm

Authors: *A. DOMONKOS[1], L. NIKITIDOU LEDRI[1], A. M. BARTH[1], M. JELITAI[1], R. KARLÓCAI[1], K. DEISSEROTH[2], T. F. FREUND[1], V. VARGA[1];

[1]Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary; [2]Stanford Univ., Stanford, CA

Abstract: Normal hippocampal operation relies on inputs from the medial septum. In turn, output from certain GABAergic neuron types of the hippocampus forms an extensive feedback to the medial septal network. These hippocampo-septal (HS) neurons receive numerous excitatory inputs from pyramidal cells, thus, they are positioned to control the septo-hippocampal connection as a function of hippocampal activity. However, there are mainly speculations about the role of the HS feedback in the regulation of hippocampal processes. In order to investigate the function of this pathway, we inactivated the HS fibers locally in the medial septum in freely behaving animals. The left and right hippocampus of somatostatin-Cre mice were transduced by a rAAV construct that Cre-dependently expresses the chloride-selective chimaeric channelrhodopsin SwiChR and YFP or only YFP as control. Optic fibers for illumination were implanted close to the medial septum, and hippocampal activity was monitored at high spatial resolution along the septotemporal axis by multishank silicon probes. In some experiments, medial septal units were also recorded using optic fiber-equipped silicon probes. The effect of HS inactivation was tested in natural sleep, linear track running, open field exploration and urethane anesthesia. Our preliminary data show that HS inactivation increases the power of theta in novel environment exploration, whereas in home cage, theta is not affected. We also characterize the effect of the HS feedback on the temporal coordination of theta across hippocampal layers and subfields as well as on the correlation pattern of concurrently recorded medial septal units.

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Poster

405. Oscillations and Synchrony: EEG studies

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EU FP7 600925 NeuroSeeker

Title: Microphysiology of the Human Alpha Rhythm

Authors: *M. HALGREN[1], J. R. MADSEN[2], D. SCHOMER[3], O. DEVINSKY[4], W. K. DOYLE[5], I. ULBERT[6], L. EROSS[7], D. FABO[8], E. HALGREN[9], S. S. CASH[10]; [1]UCSD, La Jolla, CA; [2]Departments of Neurosurg., Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [3]Dept. of Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [4]Comprehensive Epilepsy Ctr., New York Univ. Sch. of Med., New York, NY; [5]Comprehensive Epilepsy Center, New York Univ. Sch. of Med., New York, NY; [6]Inst. of Cognitive Neurosci. and Psychology, Res. Ctr. for Natural Sciences, Hungarian Acad. of Sci. and Péter Pázmány Catholic University, Fac. of Information Technol. and Bionics, Budapest, Hungary; [7]Péter Pázmány Catholic University, Fac. of Information Technol. and Bionics. Dept. of Functional Neurosurgery, Natl. Inst. of Clin. Neurosciences., Budapest, Hungary; [8]Epilepsy Centrum, Natl. Inst. of Clin. Neurosciences, Budapest, Hungary; [9]Multimodal Imaging Laboratory, Univ. of California at San Diego, La Jolla, CA; [10]Dept. of Neurology, Epilepsy Div., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: The alpha rhythm is the most ubiquitous graphoelement in the human EEG during quiet wakefulness; however, its cortical generators are still poorly characterized, especially in humans. Here, we use a combination of simultaneous macro (ECoG) and microelectrode (laminar) recordings in humans to measure which cortical layers and areas give rise to the human alpha rhythm. Although different cortical areas had distinct laminar generators of alpha activity, alpha oscillations tended to be generated in superficial and granular cell layers. Superficial layers receive diffuse matrix projections from the thalamus, whereas granular cortex receives specific, core thalamocortical afferents. This implies that thalamocortical projections play a key role in the generation of the human alpha rhythm. In some subjects, alpha phase modulated multi-unit activity in specific cortical laminae, providing unambiguous evidence that alpha exerts phasic inhibition and excitation in humans. We also discovered putative single units which were weakly (but significantly) phase-locked to ongoing and task-evoked alpha activity, directly demonstrating that alpha activity modulates neural firing. These findings provide insight into the microphysiology of the human alpha rhythm, as well as the mechanisms of how alpha phase impacts cortical processing and perception.

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Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

Title: Boosting occipital alpha power by transcranial alternating current stimulation at the second harmonic

Authors: *S. FARA[1,2], J. MCINTOSH[1,2,3], H. CHOI[1,2], C. MEHRING[1,2];
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Abstract: Transcranial alternating current stimulation (tACS) has been previously shown to interact with oscillatory brain activity. This interaction is thought to originate from entrainment of neural oscillators with an intrinsic frequency close to the applied tACS frequency, or from neuronal plasticity. To date, investigation of these hypotheses in human subjects has been limited by the technical challenge of monitoring brain activity during tACS, due to the electromagnetic artefact introduced into measurements by the stimulation itself.
Here we investigate whether tACS can increase occipital alpha power by stimulating at the second harmonic of the peak individual alpha frequency (IAF). This approach has the advantage that the stimulation artefact is localised at higher harmonics than the frequency of interest which in turn is artefact free.
Our paradigm was based on previous studies where the occipital alpha rhythm was targeted via tACS. Subjects sat in front of a monitor fixating a cross and performed an auditory attention task for a total of 45 minutes. Each participant was randomly assigned to either stimulation group (N=17) or sham group (N=15). The stimulation group received tACS with frequency equal to double the IAF, and a maximum amplitude set below each subject's perceptual and phosphene threshold. Stimulation started 5 minutes after the beginning of the experiment and lasted 20 minutes. The sham group received only 20s of stimulation. tACS electrodes were positioned over Cz and Oz on the 10-20 system. Brain activity was measured throughout the experiment by EEG. Time-frequency analysis of the EEG data showed an increase of alpha power in the stimulation group compared to the sham group, which was localized to the parietal and occipital areas. This increase was not instantaneous after the onset of tACS, but occurred over a longer time scale, becoming most pronounced in the last minutes of stimulation. Furthermore, an alpha power increase was sustained after the offset of tACS.
Consistent with previous studies demonstrating long lasting aftereffects on the alpha rhythm when stimulating at IAF, we were able to produce a similar effect by stimulating at double the IAF, suggesting that the second harmonic of IAF can interact with neural oscillators generating the alpha rhythm. Additionally, because of the absence of artefact in the frequency band of

interest we were able to observe the time course of alpha power increase during stimulation. We believe that this method could potentially be used to better understand the mechanisms underlying the interaction between brain oscillations and tACS, as well as to develop closed-loop stimulation paradigms.

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Poster

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Institute for Medical Engineering and Science, Massachusetts Institute of Technology

Title: EEG source localization of human alpha rhythms under propofol anesthesia

Authors: ***E. P. STEPHEN**[1], M. S. HÄMÄLÄINEN[3], S. KHAN[3], E. T. PIERCE[4], P. G. HARRELL[4], J. L. WALSH[4], E. N. BROWN[4,2], P. L. PURDON[4];
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Abstract: Propofol-induced unconsciousness is characterized by rhythmic electroencephalogram (EEG) activity in the slow (<1 Hz) and alpha (8-12 Hz) frequency bands. Unlike the occipital alpha rhythm present during eyes-closed wakefulness, the propofol alpha rhythm is most prominent in frontal EEG leads. Theoretical modeling studies propose that both occipital and propofol alpha are mediated by thalamocortical loops, and that the observed anteriorization arises from differential effects of propofol on occipital and frontal thalamocortical networks (Ching et al. 2010, Vijayan et al. 2013). While the propofol alpha rhythm is believed to have a cortical source in the frontal lobe, its precise anatomical source remains to be described. In this

study, we use source localization to identify the anatomical sources of the EEG alpha rhythm in humans. In particular, we perform dipole analysis and minimum norm estimates on EEG recordings from 10 healthy volunteers whose scalp EEG patterns were previously reported in Purdon et al. 2013. We find that the propofol alpha rhythm is better described by a distributed model than by a focal source. The alpha rhythm is prominent over a wide region of the frontal lobe, with the largest increases in alpha power occurring over the anterior cingulate gyrus and the dorsal and medial surfaces of the superior frontal gyrus. Using principal angle analysis with the subjects' individual neuroanatomy, we assess the ability of the analysis to distinguish between medial and lateral frontal lobe activity. Our findings provide empirical support for a model of propofol alpha that is distributed over the frontal and anterior cingulate cortices.

Ching, S, Cimenser, A, Purdon, P L, Brown, E N, & Kopell, N J (2010). Thalamocortical model for a propofol-induced α-rhythm associated with loss of consciousness. *PNAS USA*, *107*(52), 22665–22670.

Purdon, P L, Pierce, E T, Mukamel, E A, Prerau, M J, Walsh, J L, Wong, K F K, … Brown, E N (2013). Electroencephalogram signatures of loss and recovery of consciousness from propofol. *PNAS USA*, *110*(12), E1142–E1151.

Vijayan, S, Ching, S, Purdon, P L, Brown, E N, & Kopell, N J (2013). Thalamocortical Mechanisms for the Anteriorization of Alpha Rhythms during Propofol-Induced Unconsciousness. *J Neurosci*, *33*(27), 11070–11075.

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Poster

405. Oscillations and Synchrony: EEG studies

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University Research Committee, University of Cape Town, South Africa

Departmental Research Funds, Department of Psychiatry, University of Cape Town, South Africa

Title: Differentiating psychotic disorders with EEG delta and alpha band frequency and the effects of brief high frequency repetitive transcranial magnetic stimulation

Authors: *F. M. HOWELLS, J. HSIEH, H. TEMMINGH, D. J. STEIN;
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Abstract: Diagnostics within the psychotic disorders can appear to be challenging in the clinical setting. It is essential that the optimal medications are prescribed as soon as the initial psychotic episode has subsided as this provides the best prognosis to the individual that presents with a psychotic illness. Further, current treatments - antipsychotics, have been shown to ineffectively manage psychotic symptoms in up to 25% of individuals that meet the criterion for a chronic psychotic illness. We investigate whether a simple electroencephalographic (EEG) system would aid delineation of different clinical psychosis phenotypes, and whether high frequency brief repetitive transcranial stimulation (brTMS) is able to affect change, improvement, in EEG frequency activity. Specifically addressing delta and alpha frequencies as these are often highlighted in the literature to change with presentation of psychosis. In the present study we have applied a simple (6 lead montage: F_3, F_4, C_3, C_4, P3, P_4) EEG to three psychotic disorders: schizophrenia (SZ=28), bipolar I disorder with a significant history of psychosis(BPD=28), and methamphetamine-induced psychosis (MPD=24), as per DSM-IV SCID, and a socio-economic control group (CON=29). We report the data from a 3 minute resting eyes closed condition, before and after brTMS (1000 pulses at 20Hz) over the left and right dorsolateral prefrontal cortex, a total of 2000 pulses at resting motor threshold. SZ and MPD showed increased delta activity, decreased alpha, and increased delta/alpha ratios compared to controls for all 6 electrodes. MPD showed increased delta activity for parietal electrodes, decreased alpha and increased delta/alpha ratio for right hemisphere electrodes compared with BPD. brTMS showed several tendencies to further increase delta activity, decrease alpha, and increase delta/alpha ratios in SZ for left central and parietal electrodes. These data support the use of EEG frequency analysis in the delineation of psychotic disorders specifically SZ and MPD from BPD, when compared with controls. brTMS as applied in this study did not show benefit to EEG frequency activity, in fact it exaggerated the differences, specifically over the left central and parietal cortices. However, our finding of brTMS to effect change in SZ contradicts the notion of reduced plasticity, in that we saw depression in networks with stimulation where we expected to find the contrary, i.e. enhanced alpha activity and reduced delta activity.

Disclosures: F.M. Howells: None. **J. Hsieh:** None. **H. Temmingh:** None. **D.J. Stein:** None.

Poster

405. Oscillations and Synchrony: EEG studies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 405.06/H26

Topic: B.10. Network Interactions

Title: Spontaneous cortical gamma oscillation and the auditory evoked steady state response: A pharmacological investigation

Authors: *B. D. HARVEY[1], E. MOROZOVA[1], C. KELLEY[2], M. HAJOS[1,2];
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Abstract: Neurophysiological signals have been used as translational biomarkers validating functional target engagement for a variety of different drug discovery programs. Among the various neurophysiological measurements employed, auditory evoked steady-state response (ASSR) is one such biomarker that has been considered. This is particularly true for schizophrenia as it is consistently impaired in the disorder (Brenner et al., 2009) with phase locking of ASSR positively correlating with positive symptoms/hallucination. ASSR's are obtained from averaging individual responses to trains of auditory rhythmic stimuli delivered at a constant frequency; in humans the ASSR response is maximal at stimulation frequencies around 40Hz. The present study was aimed at investigating the impact of pharmacological interventions that modulate spontaneous gamma oscillations on ASSR measurements (Spencer, 2012). We tested CP-55940, a CB-1 receptor agonist and CyPPA, a SK2/3 positive allosteric modulator. These two compounds have previously demonstrated effects on broadband gamma oscillations by both increasing and decreasing spectral power respectively. Naïve C57BL/6 mice (n = 7) were chronically implanted with monopolar surface electrodes centered over frontal and parietal cortices. Each mouse was placed into an isolated recording chamber, allowed 15 minutes to acclimate after which local field potential activity (LFP) was monitored both prior-to and following presentation of auditory stimuli (30 and 40Hz click trains). This procedure was performed before and after administration of either vehicle, CyPPA or CP-55940. Spontaneous and evoked LFP data were analyzed using Matlab to calculate both spectral power and inter-trial phase coherence (ITPC). Converse to previously reported data in acute and chronic cannabis users (Skosnik et al., 2015), CP-55940 had no significant effect on the amplitude of ASSR. Similarly, CyPPA produced no significant effects on ASSR evoked power. However, analyses of ITPC showed that CyPPA significantly decreased whereas, CP-55940 significantly increased phase coherence in the frontal cortex only. Our findings indicate that ASSR phase locking but not evoked power is potentially influenced by background cortical gamma oscillations and that this should be considered when developing ASSR as a translational neurophysiological biomarker.

Disclosures: **B.D. Harvey:** None. **E. Morozova:** None. **C. Kelley:** None. **M. Hajos:** None.

Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

Support: CIHR

Title: Age-related changes in EEG during isoflurane-induced surgical vs. very deep coma anesthesia

Authors: ***P. J. SOJA**, T. MARIAM, X. DONG, R. TADAVARTY;
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Abstract: In adult rats, the cortical EEG during the surgical plane of isoflurane anesthesia (ISO, 1.0-1.5 %) is characterized predominantly by continuously occurring large-amplitude slow waves. Increasing the concentration of ISO beyond 3.5% results in a shift of EEG activity to burst events followed by suppression (*J. Neurophysiol.* 12:137-160,1949). The number of burst events eventually decrease and disappear to the point at which the EEG consists of randomly occurring single or clusters of fast, large-amplitude, sharp (FLAS) waves. FLAS waves are thought to represent a novel form of brain activity but their functional significance is not known (*Anesth. Analg.*, 79:52-57, 1994; *PLoS One* 8(9), e75257, 2013). Given that rats are commonly used to study the aging brain, the present study was performed to determine if the power in the classical frequency bands during surgical anesthesia (1.0-1.5% ISO) as well as the number of FLAS waves that characterize the EEG during very deep ISO coma (3.5%) are preserved in old age. Adult (4-6 months, n=3) and old (~18 months, n=3) male Sprague-Dawley rats were each initially anesthetized with ISO using an anesthetic chamber. The animal's trachea was intubated and its head mounted in a stereotaxic frame. Cortical EEG (S1) was recorded bilaterally using stainless steel electrodes. The concentration of ISO was adjusted to produce a stable baseline EEG over 30 min consisting of large amplitude slow wave activity. ISO was then maintained for 4 hrs while recording the EEG using CED Spike 2 software. The relative power in δ (1-4Hz), θ (5-8Hz), α (9-12Hz), and β (13-25Hz) bandwidths were analyzed with a customized script. The relative power across all bandwidths were compared using a 2 way ANOVA and Bonferroni post-test with age and time as two factors. Relative power values for δ band were significantly higher in old rats when compared to adults over a 4 hr period of ISO anesthesia whereas relative power in the θ band did not change between adult and old animals. In contrast, relative α and β power reversed between adult and old animals, being greater in adult vs. old animals during ISO.

At 1, 2, 3, and 4 hrs of very deep ISO coma, the average number of FLAS waves that occurred in 2 min epochs in adult animals measured 6, 19, 29, and 21, respectively. In contrast, in old animals, the corresponding average number of FLAS waves measured 2, 2, 2, and 3, respectively. FLAS waves in adults occurred not only as singlets, but doublets and triplets, whereas only single events were observed in old animals. These findings demonstrate distinct differences in EEG activities between adult and old animals during surgical *vs.* very deep coma anesthesia.

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Poster

405. Oscillations and Synchrony: EEG studies

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Title: Investigations of phase-amplitude coupling and long-range phase synchronization during multisensory working memory maintenance - an MEG study

Authors: *J. DAUME[1], T. GRUBER[2], A. K. ENGEL[1], U. FRIESE[1];
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Abstract: Working memory (WM) maintenance has been associated with neuronal oscillatory activity over a broad range of frequencies. Recent work showed that the phases of lower frequencies modulate amplitudes of higher frequencies and this phase-amplitude coupling (PAC) has been suggested to serve as a key mechanism for WM storage. Moreover, long-range phase synchronization within low frequency bands have been linked to WM control processes. Most studies, however, investigated such interactions in a unisensory environment. Here, we aimed at examining how divided resources during multisensory WM affect amplitudes, PAC and phase synchronization of oscillatory activity when compared to unisensory WM processes. We used magnetoencephalography to record neural activity of healthy human participants engaged in an audiovisual delayed-match-to-sample task. In each trial, participants were stimulated twice with a simultaneous presentation of a picture and a sound. Their task was to judge whether stimuli

were the same or different within the modalities. To this end, visual and auditory stimuli were always modified independently from each other. In one condition, participants had to focus on the visual stimulus only, while in the other, responses for both the visual and the auditory stimuli were required. This setup allowed us to compare unisensory WM processes against those where resources were distributed across sensory systems. As expected, in both conditions sensory regions showed elevated beta and gamma as well as decreased alpha power throughout the delay period. Frontal areas predominantly exhibited increased theta power. Differences in power between the conditions were apparent in frontal regions, where multisensory WM led to more theta power. Further, a cluster of occipital sensors showed stronger alpha-gamma PAC during periods of distributed resources. Source estimates located this effect in right extrastriate area V3. Comparing imaginary coherence between the conditions within the alpha band revealed weaker long-range phase synchronization between right and left V3 during audiovisual WM. Our results provide insights into interactions of neuronal oscillations during multisensory WM maintenance. They suggest that stronger alpha-gamma PAC and weaker alpha-band phase coupling within the visual system may be a neural correlate of increased task demands when resources are distributed between sensory systems that are engaged in WM maintenance.

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Poster

405. Oscillations and Synchrony: EEG studies

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Title: Dopaminergic medication restores abnormal theta-band neural synchronies during a working memory task in patients with restless legs syndrome

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Abstract: Restless legs syndrome (RLS) is a sensorimotor neurological disorder which is characterized by an irresistible urge to move one's legs. Dopaminergic agonists play a role in improving cognitive function in RLS patients, but its neural mechanism remains unknown. In

this study, to identify the electrophysiological evidences of the effect of dopaminergic medication on cognitive function in RLS patients, we investigated interregional neural synchronies during a working memory task.13 normal controls and 13 drug naïve RLS patients participated in the study. Two event-related potential (ERP) recordings were performed; the first was just before giving the first dose of pramipexole (baseline condition) and the second was at 12 - 16 weeks after commencement of pramipexole administration (follow-up condition). Subjects performed a Sternberg working memory task. At encoding phase, a series of digit number was presented after visual orienting cue sign. After 2-s maintenance interval, a probe item was shown at retrieval phase. Subjects were required to press a button if the probe corresponded to one of the number shown in the encoding phase. During the task, 19 channel electroencephalograms (EEGs) were recorded. We analyzed the event-related interregional phase synchrony in theta-band (TBPS) during retrieval phase. The spatial pattern of TBPS was quantitatively analyzed using graph theoretical measures, including clustering coefficient (C) and characteristic path length (L). At 400-700 ms after the prove onset, considerable increment of TBPS was observed mainly between anterior and posterior regions for all subjects. At this period, the anterior-posterior TBPS for RLS patients in baseline condition remarkably weaker compared to normal control, but recovered after the medication. RLS patients in baseline condition showed significantly reduced C and increased L compared to normal controls. However, this alteration for RLS patients was significantly recovered to the normal level after the medication. In sum, we observed significant alterations of interregional neural synchronies and inefficient brain networks for RLS patients, which may underlie the deficit of working memory function associated with RLS. Also, theses abnormalities were restored after dopaminergic medication. Our findings imply that dopamine plays a major role in the cause and recovery of cognitive deficit in RLS patients.

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Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

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Title: High frequency oscillations evoked by tones in auditory cortex

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Abstract: Electrocorticographic (ECoG), signals in the 70-200 Hz range, variously called broad band "high frequency" (HF) or "high-gamma" (HG), have a broadband power distribution that is generally believed to reflect massed multiunit neuronal firing in neuron populations near the recording electrode. It is often assumed that ECoG signals in the this range do not contain oscillatory signals, though earlier intracortical recordings in monkeys and scalp EEG recordings in humans report that high frequency oscillations (100-200 Hz) can be evoked by intense, rapid onset auditory and visual stimuli.
We recorded field potential (FP) and multiunit activity (MUA) responses to tones in the macaque auditory cortex, using linear-array, multielectrodes, and addressed the laminar pattern of HG activity. FPs and MUA in auditory cortex responded to pure tones (100 ms duration, 60dB) at an average onset latency of about 10 msec. In subsets of cortical sites, the FP onset response to the local best frequency tone was followed by a transient rise in the amplitude of HG activity, whose instantaneous frequency changed dynamically from about 200 Hz to 80 Hz over a 40-70 ms period, beginning at 30 ms from tone onset. Current source density (CSD) analysis of the FP profile revealed that the laminar extent and distribution of this HF activity varied across recording penetration sites with a bias toward the granular and supragranular layers. CSD analysis also showed that the neuronal generators of the HF response are oscillating current source/sink configurations that clearly replicate the dynamic shift from ~200-80 Hz from 30-100 ms poststimulus, observed in the FPs. These results indicate that tone-evoked HG activity in auditory cortex is generated by a spatially delimited, current dipole oscillating at the HG frequency. Dependence of the HG amplitude on the tone frequency showed spectral tuning of HG was similar to concomitant MUA. However, no apparent relationship between HG phase and MUA was observed.

Disclosures: Y. Kajikawa: None. **C.E. Schroeder:** None.

Poster

405. Oscillations and Synchrony: EEG studies

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Title: Abnormal cortical rhythm and network during rapid-eye movement sleep in patients with rapid-eye movement sleep behavior disorder

Authors: *S. HEO[1], H. KIM[1], B.-U. LEE[2], S.-A. KU[2], J.-I. BYUN[3], J.-S. SUNWOO[4], K.-Y. JUNG[2], K. KIM[1];
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Abstract: Rapid-eye movement (REM) sleep behavior disorder (RBD) is a sleep disorder characterized by dream enactment behavior and the loss of muscle atonia during REM sleep. In this study, we compared functional cortical networks during REM sleep between healthy controls and RBD patients, in order to investigate abnormal changes of neural synchrony in RBD patients. Drug-naïve idiopathic RBD patients and healthy controls were participated in the experiment. 21 channel electroencephalograms (EEGs) were recorded during one overnight sleep. Artifact-free 30s epochs in REM sleep stage were analyzed. In order to examine the differences in local and global neural synchronies between two groups, time-varying spectral power and inter-regional phase synchronizations (PS) were investigated. The spatial characteristics of the pattern of PS were quantified by graph theoretical measures. Significantly increased power and centroparietal connection were observed in delta-band for RBD patients compared to normal controls. In RBD patients, the number of connections at delta-band were anti-correlated with Korean version of the sniffin' sticks test (KVSS) score, which is known as a good index of the severity of neurodegenerative disease. Significantly reduced clustering coefficient and increased characteristic path length were observed for the RBD patients compared to normal controls. The small-worldness propensity, which represents the network efficiency, was significantly reduced for the RBD patients compared to normal controls in delta- and alpha-bands. The increments of delta- and theta-bands power during REM sleep were known as EEG slowing activity, which reflects neurodegeneration in the neurological patients. The abnormally increased neural synchronies in our results can be understood as reflecting the neurodegenerative process associated with RBD. The alteration in the functional cortical network in delta and alpha bands reflects loss of the small-world network characteristics in the RBD patients. The abnormal subband power and functional connectivity may serve as EEG-based neuromarkers for the RBD.

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Poster

405. Oscillations and Synchrony: EEG studies

Location: Halls B-H

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Topic: B.10. Network Interactions

Support: NIH intramural research program

Title: Stability of timing and connectivity in functional networks of the human cortex

Authors: *J. CHAPETON, S. K. INATI, K. A. ZAGHLOUL;
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Abstract: Despite many advances in the study of large-scale functional networks, the question of timing, stability, and direction of communication between cortical regions has not been fully addressed. We hypothesized that if two brain regions are communicating, the activity of one should predict the activity of the other with a consistent time delay. Although such predictive relationships have been identified between neurons using measures of effective connectivity, it is not known whether similar relationships exist between cortical regions. We examine this question here using resting-state intracranial EEG captured from nine human participants with medically refractory epilepsy. We used a coupling measure based on time-lagged mutual information to identify effective connections between brain regions that exhibit a statistically significant increase in average mutual information at a consistent time delay. We constructed functional networks based on these connections and found that individual connections and overall network topology were stable over minutes, hours, and days. Notably, the time delays associated with these connections were also highly preserved over multiple time scales, suggesting the presence of stable functional networks in the human brain. We identified individual brain areas that exhibited stronger homogeneity in their connections across data blocks; there was no clear anatomic distribution of these stable nodes. However, we found that a node's homogeneity across time was strongly correlated with its degree and clustering coefficient, suggesting that network stability is in large part mediated by highly connected nodes and their partners. We characterized the spatial properties of information flow through these functional networks, and found a preferred posterior to anterior temporal lobe direction, consistent across all participants. Our results demonstrate that cortical regions exhibit functional relationships with well-defined and stable timing, and suggest that connectivity and time delays between brain regions are highly consistent across multiple time scales.

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Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

Support: The GIST Research Institute (GRI) in 2016.

Title: EEG connectivity analysis for healthy adults under anesthesia

Authors: *J. CHOI[1], S. AHN[1], H. CHO[1], M. KWON[1], S. LEE[1], S. JANG[1], B.-M. CHOI[2], G.-J. NOH[2,3], S. JUN[1];
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Abstract: Electroencephalography (EEG) is commonly used to measure the depth of anesthesia. Clinical signs and EEG patterns during general anesthesia is well understood. There exist many reports about connectivity diminishment between the frontal and parietal areas by loss of consciousness. Propofol is a typical GABAergic anesthetics which disrupts the fronto-parietal network. In this study, we tested the hypothesis that propofol would inhibit cortical connectivity in frontoparietal networks using MATLAB connectivity toolbox "HERMES".
Healthy subjects (n=15) were recruited for induction of anesthesia with intravenous propofol (12mg/kg/h); EEG data with 6 channels (Fp1, Fp2, F3, F4, P3, and P4) were acquired. For every subject, resting state EEG data (as a baseline) were acquired for about 5 minutes. The anesthetic was administrated during one hour. EEG data were collected during administration and for 60 minutes after the end of administration. EEG data of each subject were segregated into three states of consciousness (Baseline (resting), Loss of Consciousness (LOC), and Recovery) We measured correlation (COR), coherence (COH), imaginary part of coherence (iCOH), and phase-locking value (PLV) for connectivity analysis between frontal (4 channels) and parietal (2 channels) areas.
By the administration of propofol, the fronto-parietal connectivity was significantly ($p < 0.01$) inhibited during LOC. Average COR decreased from 0.64 to 0.49, COH also diminished from 0.41 to 0.25. iCOH was significantly inhibited since iCOH value of unconscious state was quite lower than that of baseline state. PLV between baseline and unconscious state also showed significant difference, but was relatively inferior to other methods. These results were tabulated in Table 1. Connectivity difference between baseline and recovery states also showed significance for all three measures. However, PLV between LOC and recovery states was not significant ($p = 0.074 > 0.01$).
Four EEG connectivity measures for healthy adults under general anesthesia process were

compared. Every measure detected the disruption of frontal-parietal communication, despite PLV yielded relatively weak connectivity difference between consciousness states.

Connectivity comparison between basline and loss of consciousness state

		COR	COH	iCOH	PLV
Mean±SD	BSL	0.64±0.0991	0.4135±0.1544	0.0282±0.0104	0.5786±0.1218
	LOC	0.4989±0.0936	0.2465±0.0826	0.0076±0.0029	0.4448±0.0648
p-Value		0.000034	0.000037	0.000001	0.00018

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Poster

405. Oscillations and Synchrony: EEG studies

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Title: Inter-brain connectivity during live verbal interaction

Authors: ***S. AHN**[1], H. CHO[1], M. KWON[1], K. KIM[2,3], B. KIM[4], W. CHANG[5], J. CHANG[5], S. JUN[1];
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Abstract: Investigation of an inter-brain synchronization during social interaction has gained significant attention. One EEG study attempted to address inter-brain coordination when two subjects were engaged in verbal communication, and oscillatory modulations in the theta and alpha bands were reported. However, there are only few studies on verbal interaction and its significant inter-brain connections are still under investigation. In this work, therefore, we performed reciprocal live verbal interactions between humans, and collected EEG data to investigate intra- and inter-brain functional connections. Nine males and one female (five-pair) participated in live verbal interaction. Every randomly paired subjects performed the experiments in two different magnetically and electrically shielded rooms, one room for each. Distance between two shielded rooms is about 100 miles. Nineteen channels EEG system was used. Subjects were instructed to interact only verbally with their partner by counting the number from 1 to time limit. For the first 5 seconds, instruction was displayed in the screen and subject prepared to do given task with yellow characters and black background. After this instruction period, a task period was begun with black blank screen during 30 seconds. There are two tasks in this task period. First task is interaction task (INT) which each subject performed turn-taking number counting from 1 to time limit (30 seconds). Second task is speaking task (SPE) which each subject counts the numbers from 1 to time limit for oneself. After task period, break instruction was displayed for 5 seconds. Each subject stops the counting and prepares for next trial in this period. Instruction, task and break period are 1 trial and duration is 40 seconds. It consisted of 12 trials per run and 5 runs were obtained. For connectivity analysis, we adopted weighted phase lag index to minimize the effect of volume conduction. With a thousand surrogated data, the statistical significance of functional connectivity was estimated. As a result, a strong functional intra- and inter-connections were observed between left temporal and right centro-parietal lobes in EEG alpha band (8-12Hz) during INT condition compared to SPE. Right centro-parietal lobe plays an important role in social interaction and it is called phi-complex as a neuromarker of human social coordination. In addition, left temporal lobe (left superior temporal gyrus) is in charge of auditory perception and manipulation as following ventral stream of the brain. These neurophysiological evidences support our findings which is significant functional connections in two specific brain regions.

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Poster

405. Oscillations and Synchrony: EEG studies

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Topic: B.10. Network Interactions

Support: BIAL 220/12

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Title: 0.0002 Hz fluctuations in human intracranial DC recordings

Authors: *G. PIANTONI[1,2], E. S. ROSENTHAL[1,2], E. HALGREN[3], S. S. CASH[1,2];
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Abstract: Neural activity is organized in rhythms at multiple temporal scales, from the circadian to the ultra-fast oscillations. While the electroencephalography commonly records oscillations in the range of 0.1 to 100 Hz, the presence of very slow fluctuations has been difficult to assess due to the widespread use of hard-pass filters.
In this study, recordings were acquired with a full-band amplifier in intracranial electrodes implanted for clinical mapping of the epileptogenic zone in patients with intractable epilepsy, continuously over the course of a few days. We measured the difference in DC potentials between neighboring channels belonging to depth electrodes in prefrontal and temporal cortices. This setup prevents the potential confound that our findings are affected by changes in skin conductance that have limited previous DC studies.
We show, for the first time to our knowledge, the existence of fluctuations with a period of 1-2 hours in the human brain. The ultra-slow oscillations were of an amplitude in the order of mV, which is considerably larger than previously reported brain rhythms, and were present during both wakefulness and sleep. Crucially, the cycle of the ultra-slow fluctuations was correlated with the sleep cycle, especially at the beginning of the night. This observation was confirmed by the cross-frequency coupling which was present exclusively during sleep: the phase of the ultra-slow fluctuations entrained the power in the slow wave band (~1 Hz).
In conclusion, we show that the human brain generates ultra-slow fluctuations of very large amplitude in the order of 1-2 hours. These ultra-slow fluctuations synchronize the sleep cycle and their phase might indicate the propensity to fall asleep. We speculate that these fluctuations represent a potential correlate of the Basic Rest-Activity Cycle (BRAC), a putative rhythm that organizes the level of vigilance during wakefulness and the sleep cycle during sleep.

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Poster

405. Oscillations and Synchrony: EEG studies

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MGH Scholars Fund

Title: Neural network dynamics during recovery from isoflurane as revealed by electroencephalogram in young and old rats

Authors: *B. F. COUGHLIN[1], S. S. CASH[2,3], E. Y. KIMCHI[2,3];
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Abstract: General anesthesia is ubiquitous in invasive surgical procedures. However, despite widespread use of anesthesia, the neural network dynamics of the anesthetized state remain unclear, particularly during recovery. Additionally, both older patients and older animal subjects recover more slowly from anesthesia, as defined by behavioral measures. The global oscillatory dynamics during recovery from anesthesia have neither been comprehensively described nor extensively compared between older and younger subjects. To characterize the neurophysiologic signatures of recovery from anesthesia and compare them between older and younger subjects, we recorded EEG and EMG from young (aged 2-4 months, n=15) and old (aged 22-24 months, n=13) rats during and following 2% isoflurane anesthesia. EEG and EMG recordings during two hours of isoflurane anesthesia revealed burst-suppression in all subjects. Recordings were then continued after the conclusion of isoflurane anesthesia through behavioral recovery, as defined by the return of righting reflex (RORR). As there are currently no well-defined electrographic stages of recovery from anesthesia, we first qualitatively characterized EEG and EMG changes during recovery. Spectral representations of EEG and EMG recordings revealed a consistent progression of several features or states during the recovery from isoflurane anesthesia, including transitions from broadband activity following burst suppression to a combination of delta, slow theta, and beta band activity. Correlation analyses were performed to determine relationships between features. Interestingly, younger animals appeared to remain in post-anesthesia burst suppression longer than older animals, but ultimately recovered their righting reflex more quickly. Higher frequency EEG and EMG activity appeared to predict the return of righting reflex in all subjects, but with particular distinctions in young and old subjects. While quantitative analyses are ongoing, it is our hope that the identified neurophysiologic states can serve as markers of recovery and can begin to lay the groundwork for a more-complete picture of the course of anesthetic recovery and the neural networks involved.

Disclosures: **B.F. Coughlin:** None. **S.S. Cash:** None. **E.Y. Kimchi:** None.

Poster

405. Oscillations and Synchrony: EEG studies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 405.17/I11

Topic: B.10. Network Interactions

Support: European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement nr. 600925

Title: Characterization of networks for rule processing in human stereoEEG data using consensus-based partial coherence

Authors: ***M. TER WAL**[1,2], P. CARDELLICCHIO[3], G. A. ORBAN[3], P. H. TIESINGA[1,2]; [1]Neuroinformatics, Radboud Univ., Nijmegen, Netherlands; [2]Donders Inst., Nijmegen, Netherlands; [3]Dept. of Neurosci., Univ. of Parma, Parma, Italy

Abstract: The demands for sensory processing are dependent on context. To study context related changes in visual processing, we set up a task in which the visual stimulus was identical throughout the experiment, while the desired output changed between two rules: color and orientation. Throughout the experiment we recorded local field potential signals in epilepsy patients that were admitted for presurgery evaluation and had cylindrical stereo-electroencephalography (sEEG) electrodes implanted across frontal brain regions in one or both hemispheres.

One approach to characterize functional networks in sEEG is to compute pair-wise coherence between channels, an undirected measure of spectral covariance, which is computationally efficient. However, pair-wise coherence is confounded by shared inputs to the studied pair: a high coherence may be obtained, even in the absence of an underlying connection. The coherence can be corrected for shared inputs by conditioning on the data from all other simultaneously recorded channels, yielding the so called partial coherence [1]. Calculating the partial coherence involves inverting the n-by-n cross-spectrum matrix, where n is the number of recorded channels. Partial coherence can perform well for datasets with a few channels, but for sEEG data, with its high channel count, the matrix becomes ill-conditioned, preventing accurate estimation of its inverse.

Here, we present a solution to this conditioning problem. Instead of conditioning on the full dataset, channels are pooled into a small number of groups and conditioning is performed on the set of group-averages. By repeating this procedure for different random groupings of channels, a reliable consensus is reached for the partial coherence of a pair. We first validated this approach

for a range of conditions in model data. The modeling results confirmed that partial coherence removed shared inputs from the network characterization, while maintaining direct connections. Subsequently, we demonstrated that consensus-based partial coherence can be used to identify networks in sEEG data recorded in epilepsy patients. Here too, it reduced the identified networks for rule processing, in a time and frequency resolved way. Taken together, this new methodology allows for assessing functional brain networks in large data sets.
[1] R. Rosenberg, M. Halliday, P. Breeze, and A. Conway, "Identification of patterns of neuronal connectivity—partial spectra, partial coherence, and neuronal interactions," J. Neurosci. Methods, vol. 83, pp. 57 – 72, 1998.

Disclosures: **M. Ter Wal:** None. **P. Cardellicchio:** None. **G.A. Orban:** None. **P.H. Tiesinga:** None.

Poster

405. Oscillations and Synchrony: EEG studies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 405.18/I12

Topic: B.10. Network Interactions

Title: Cross-frequency coupling: the theoretical framework.

Authors: *G. CISOTTO;
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Abstract: Increasing attention is being given to quantify the so-called cross-frequency coupling (CFC), that is the relationship between brain activities at different frequencies. Particularly, recent empirical evidence shown that neuronal activity at low frequency, e.g. theta band, modulates with its phase changes the magnitude of neuronal activity at higher frequency, e.g. gamma band, in the same area or in another one, functionally connected with the first one. Several methods have been employed to quantify this phenomenon in different kinds of electrophysiological signals. Unfortunately, several parameters had to be a priori determined: the frequency and the bandwidth of the two components interacting in the CFC, above all. Their selection has been most often data-driven. Moreover, the output of such methods is usually represented by an index that is supposed to quantify the strength of the coupling. However, no realistic range for it exists, unless a very conservative [0,1] range that has already been shown to be overestimated, being the most common empirical values in the order of 0.001. This contribution aims at translating the well-known theoretical framework about signal modulation from its original field of communication engineering to the study of CFC. Thanks to it, it was possible to limit the range of the most important parameters in order to ensure a proper coupling.

Besides, a common driver for the two signals - a third one at a much lower frequency - could be also extracted, providing support for the hypothesis of a common generator of the CFC mechanism. A simulator for the CFC was designed as the parallel modulation in phase (PM) of a low frequency signal xL(t) and in amplitude (AM) of a high frequency one, xH(t). Their coupling was given by a third signal a(t) that simultaneously drove the phase changes of xL(t) and the amplitude variations of xH(t). Using this controlled environment, it could be possible to test the variation of CFC strength produced by changing the different parameters of interest. Optimal demodulator for decoding CFC from a typical CFC signal was designed as well. The strength of CFC was computed by the Pearson's correlation coefficient ρ between the two signals obtained at the demodulator output from the low and high frequency components, respectively. Thus, the optimal choice of parameters could be identified as the combination at which a maximum ρ is achieved. This contribution provides a platform (i) to simulate an expected strength of CFC, (ii) to test the effectiveness of a specific standard method of quantification of CFC and (iii) to optimally demodulate both PM (xL(t)) and AM (xH(t)) components to check if the common generator a(t) exists.

Disclosures: G. Cisotto: None.

Poster

405. Oscillations and Synchrony: EEG studies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 405.19/J1

Topic: B.10. Network Interactions

Support: Vladimir Visocky is supported by a BBSRC funded Case PhD studentship with AstraZeneca

Title: Site-dependent effects of optogenetic stimulation in thalamic reticular nucleus on cortical states

Authors: *V. VISOCKIS[1], S. SAKATA[1], B. MORRIS[2], J. PRATT[1];
[1]Strathclyde Univ., Glasgow, United Kingdom; [2]Glasgow Univ., Glasgow, United Kingdom

Abstract: The corticothalamic loop has long been implicated in the range of neuropsychiatric diseases. The thalamic reticular nucleus (TRN), a part of the corticothalamic loop, plays a key role in selective attention and sleep spindles. Furthermore, sleep spindles are reduced in amplitude and duration in schizophrenia patients, implying clinical relevance of TRN functions. However, while the TRN is topographically organized, it still remains unclear whether and how the TRN consists of functionally distinct sub-regions. Combining optogenetic and

electrophysiological approaches in mice, we investigated changes in sleep spindles and EEG oscillations caused by optogenetic stimulations in different parts of the TRN. Archaerhodopsin (Arch), a light sensitive proton pump, was expressed specifically in either an anterior or posterior part of the TRN in parvalbumin (PV)-Cre mice using adeno-associated viral vectors. We found restricted expression patterns of Arch in PV-positive neurons of the TRN depending on injection sites. Effects of optical stimulation on cortical EEGs were assessed by delivering green light through chronically implanted optic fibers over up to 1 min periods in freely behaving animals. Tonic stimulations during awake states did not produce any significant change in EEGs whereas stimulations during sleep modulated delta power and the number of sleep spindles. Together these data support the notion that activity in the TRN may have different impacts on the modulation of cortical states in a site-dependent manner.

Disclosures: **V. Visockis:** None. **S. Sakata:** None. **B. Morris:** None. **J. Pratt:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 406.01/J2

Topic: B.11. Epilepsy

Support: NIH R01 NS082046

Title: Contrasting properties of hippocampal dentate gyrus and CA1 principal cells

Authors: ***S. A. PARK**[1], D. A. COULTER[1,2,3];
[1]Neurol., [2]Pediatrics, Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The hippocampus is known to be involved in spatial learning and navigation, where each region behaves differently and performs distinct functions. In vivo, dentate granule cells (DGCs) are less responsive to afferent stimulation compared to CA1 pyramidal cells (CA1 PCs), which we also demonstrated in vitro by imaging the expression of the fluorescent, activity dependent label CaMPARI in DGCs and CA1 PCs in horizontal hippocampal slices in response to afferent stimulation. Using current-clamp, we explored intrinsic and synaptic properties that could underlie the variable responsiveness of the two cell types. We identified several intrinsic properties that could contribute to DGCs being less excitable than CA1 PCs. DGCs had a larger input resistance, more hyperpolarized membrane potential, and a higher voltage threshold for generating the 1st action potential compared to CA1 PCs. However, DGCs also had lower membrane capacitance and less dendritic branching. We also recorded aggregate synaptic

responses composed of EPSPs and IPSPs in current-clamp mode in response to a threshold afferent stimuli, and calculated the response amplitude at times corresponding to peak activation. DGCs were harder to excite as well as inhibit synaptically compared to CA1 PCs. The peak DGC EPSP (3.3 nS) and IPSP (3.2 nS) conductances were both smaller compared to CA1 EPSP (8.2 nS) and IPSP (6.8 nS), respectively, indicating lower levels of activation of DGCs by threshold stimuli. Additionally, our data show that in CA1 PCs, IPSPs follow EPSPs and are sustained for at least 100 ms post-stim, whereas the majority of DGC EPSPs reach peak earlier than CA1 EPSPs and are smaller in magnitude, with no apparent early or delayed IPSP, which suggests that the DGC response was an aggregate, extensively overlapping EPSP/IPSP, which dampened excitation. Overall, our data show DGCs are harder to excite and temporal overlap in synaptic inputs may be a primary mechanism determining DGCs' reduced synaptic excitability compared to CA1 PCs. Continued investigation of synaptic connections and microcircuit properties in cells with identified aggregate responses may further clarify how each hippocampal cell type processes synaptic input to generate their regionally characteristic behavior.

Disclosures: **S.A. Park:** None. **D.A. Coulter:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: NIH R01-NS031718-17 (FEJ)

NIH 1DP10D00347 (FEJ)

NIH IDDRC P30 HD 18655 (FEJ)

CIHR MFE-115462 (HS)

Title: Impairment of homeostatic synaptic scaling in epileptic immature brain

Authors: *H. SUN, D. TALOS, F. E. JENSEN;
Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Synaptic scaling stabilizes network excitability by adjusting synaptic strength in response to chronic global changes in neuronal firing. Synaptic scaling is most robust in neurons during early development, and our previous study has shown a PLK2-mediated homeostatic compensation following early-life seizures. However, this homeostatic downregulation of

excitability was incomplete and negatively regulated by co-activation of mTOR pathway. Here, we aimed to determine the capability of neurons in epileptic immature brain to generate homeostatic synaptic scaling. Early-life Seizures (HS) were induced by acute global hypoxia in P10 rats. *Ex vivo* slices were maintained *in vitro* for 48-72h using an acute culture procedure. Whole-cell clamp recordings were made in cortical pyramidal neurons of somatosensory cortex from post-HS 48h rats and littermate controls. Human epilepsy biopsy tissues were collected following surgical resection for medically intractable epilepsy. We found that at 48-72hrs post-HS, the spontaneous firing rate was significantly higher in neurons from post-HS rats (0.35±0.036 Hz, n=9) compared to normoxic controls (0.11±0.026, n=7, $p<0.001$). Chronic activity blockade by 0.001mM TTX for 24-48h induced significant increases in amplitude and frequency of AMPAR mEPSCs in neurons from both HS rats (Amplitude: 12.3±0.5pA vs 16.6±0.8pA, n=6-8, $p<0.05$; Frequency: 100±15.2% vs 151.2±18.2%, n=6-8, p=0.0507) and normoxic controls (Amplitude: 10.3±0.7pA vs 12.8±0.6pA, n=7-8, $p<0.05$; Frequency: 100±13.2% vs 161.2±21.4%, n=7-8, $p<0.05$) with no changes in AMPAR mEPSC rising and decay time (all $p>0.05$, n=7-8). However, the decreases in synaptic strength in response to elevated synaptic activity by application of 0.1μM Picrotoxin 24-48h as identified in controls (Amplitude: 8.26±0.59pA, n=7, $p<0.05$; Frequency: 83.59±15.67%, n=7, p=0.433) was occluded in neurons from post-HS rats (Amplitude: 13.29±0.49pA, n=8, p=0.179; Frequency: 93.57±20.95%, n=8, p=0.807). In human epilepsy biopsy cortical slices, synaptic scaling down in response to 48h elevated synaptic activity by application of Picrotoxin was abolished (AMPAR mEPSC amplitude: 9.4±0.3pA vs 9.7±0.4pA, n=6-8, $p>0.05$; Frequency: 100±10.2% vs 163.1±13.2%, n=6-8, $p<0.05$), while scaling up in response to 48h synaptic activity blockade by TTX was remained (AMPAR mEPSC amplitude: 11.8±0.6pA, $p<0.05$; Frequency: 112.6±12.9%, $p>0.05$, n=7). These data suggest an impaired synaptic scaling down in cortical neurons in epileptic immature brain, which may, at least in part, contribute to neonatal seizure-induced long-term neuronal hyperexcitability and epileptogenesis.

Disclosures: H. Sun: None. **D. Talos:** None. **F.E. Jensen:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Program#/Poster#: 406.03/J4

Topic: B.11. Epilepsy

Support: NIH NINDS RO1 NS083402

Title: Effects of de novo epilepsy encephalopathy mutations of KCNQ2 on voltage-dependent activation of Kv7/KCNQ channels via their interaction with CaM

Authors: *E. KIM[1], S. WANG[1], W. PANG[1], J. CAVARETTA[1], H. CHUNG[1,2];
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[2]Univ. of Illinois at Urbana Champaign, Neurosci. Program, Urbana, IL

Abstract: Kv7/KCNQ channels composed of KCNQ2 and KCNQ3 subunits are voltage-gated potassium channels, underlying the neuronal M-current (I_{KM}), that potently limits repetitive and burst firing of action potentials in neurons. Consistent with their ability to inhibit excitability, they are preferentially enriched at the plasma membrane of the axon initial segment (AIS) and axon. Mutations in KCNQ2 have been associated with a wide spectrum of early-onset epileptic disorders ranging from benign familial neonatal seizures (BFNCs), an autosomal-dominant epilepsy of newborns, to severe epileptic encephalopathies (EEs). Some EE mutations are found in the calmodulin (CaM)-binding regions of intracellular carboxy-terminal tail of KCNQ2 (R333W, M518V, R532W and K526N). We found that these EE mutations on the KCNQ2 C-terminal tail variably disrupted CaM binding to KCNQ2. To test the extent to which EE mutations disrupt voltage-dependent outward K^+ current through KCNQ channels, we performed whole-cell voltage clamp recording in CHOhm1 cells. We found that that expression of R333W and M518V mutants showed much smaller voltage-dependent outward K^+ current and current density than expression of wild-type KCNQ2. Interestingly, KCNQ2-K526N mutant channels opened at less depolarizing potential than the wild-type KCNQ2 channels. Our results indicate that the EE mutations reduce KCNQ current by disrupting KCNQ2 interaction with CaM binding. These current reductions in combination with decreased axonal surface expression and protein stability of KCNQ channels (Cavaretta et al., poster) would cause severe defects in the ability of KCNQ channels to inhibit neuronal hyperexcitability, ultimately leading to epilepsy.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 406.04/J5

Topic: B.11. Epilepsy

Support: NIH/NIGMS Grant 5T32GM008328-24

Title: Low KCC2 expression in reticular thalamic neurons is sufficient to regulate network activity

Authors: *P. M. KLEIN, M. E. HARPER, H. M. MCKOWN, M. P. BEENHAKKER;
Univ. of Virginia, Charlottesville, VA

Abstract: Large-scale thalamocortical network oscillations underlie seizures associated with absence epilepsy. While GABAergic signaling among reticular thalamic (RT) neurons likely suppresses hypersynchronous thalamic activity, it remains unresolved whether this desynchronizing mechanism occurs through excitatory or inhibitory transmission. Inhibitory GABAergic signaling has long been thought to depend on Cl^- extrusion by chloride cotransporter proteins, including KCC2. Yet previous studies report virtually nonexistent KCC2 expression among RT neurons, thus supporting a hypothesis that GABAergic signaling exerts a depolarizing effect. However, this conflicts with both the existing thalamocortical circuit models and our own finding that GABA inhibits RT neurons. Therefore, we aim to determine the diverse mechanisms that regulate thalamic Cl^- homeostasis and GABAergic signaling. While our immunohistochemical staining from P10-40 Sprague Dawley rats indicates KCC2 levels are indeed significantly reduced in RT relative to other thalamic regions (P10: 70±3.3%, P20: 68±2.7%, P40: 62±4.1%, $p<0.001$ and $n=4$, normalized to ventrobasal thalamus expression), RT still expresses moderate, functionally relevant KCC2 levels. We then measured the GABA reversal potential (E_{GABA}) of RT neurons and show that it supports inhibitory GABAergic signaling (E_{GABA}: -62±3.1 mV, $n=13$). The KCC2 antagonist VU-0463271 depolarizes the E_{GABA} of RT (ΔE_{GABA}: +16±5.4 mV, $p=0.003$, $n=5$), confirming its functional relevance.Impermeant anion distribution, both within and surrounding neuronal somata, are also reported to regulate intracellular Cl^- concentrations. We have determined that sulfated proteoglycans, components of the extracellular matrix and a major source of extracellular impermeant anions, become significantly upregulated in RT by P15 (P10: 113±6.4% $p=0.064$, P15: 132±5.1% $p<0.001$, P40: 172±12.3% $p<0.001$, $n=4$ for all). These extracellular impermeant anions appear to contribute to Cl^- homeostasis in RT.As we have identified a critical role of KCC2 in regulating GABAergic signaling among RT neurons, we sought to determine how this mechanism regulates absence seizures. Alteration of KCC2 activity in the thalamus of WAG/Rij rats, through targeted microdialysis infusions, has allowed us to observe the role of this important Cl^- cotransporter on spontaneously occurring absence seizures. Similar changes in network activity of thalamic slices was observed with functional calcium imaging. By clarifying the mechanisms that regulate thalamic oscillatory activity, we hope to provide new targets for treating absence epilepsy.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: National Science Foundation DGE-1333468

Epilepsy Research UK (P1402)

Title: Cardiac and respiratory consequences of repeated epileptic seizures in rat

Authors: D. PEDERSON[1], A. ASHBY-LUMSDEN[3], P. P. IRAZOQUI[2], *J. G. JEFFERYS[4]; [1]Biomed. Engin., [2]Weldon Sch. of Biomed. Engin., Purdue, West Lafayette, IN; [3]Pharmacol., [4]Univ. of Oxford, Oxford, United Kingdom

Abstract: Sudden death in epilepsy (SUDEP) is a significant and devastating risk in some kinds of epilepsy. The most likely explanations are respiratory or cardiac failure during or following a seizure. To determine the impact of recurrent seizures on cardiac and respiratory function we induced temporal lobe epileptic foci by injecting tetanus toxin (5-10 ng in 400 - 1000 nl) into rat hippocampus. Spontaneous seizures started a few days later, each lasting <2 min. Status epilepticus did not occur at any stage. We implanted wireless telemetry devices (either a subcutaneous Bionode developed and manufactured at Purdue, or an intra-abdominal Millar TR50BB). Leads were tunnelled subcutaneously to ECG and ECoG electrodes and, in the case of the Bionode, to a thermocouple implanted in the nasal passage. Telemetry and video recordings were made for <6 weeks after injection, and in some cases for 1-2 weeks before injection. Heartrate increased during nearly all seizures and remained high for minutes to tens of minutes afterwards. This postictal tachycardia could occur when the rat was completely inactive and was exacerbated by postictal physical activity. Arrhythmias were common during secondarily generalised seizures and included missed beats, asystoles, premature ventricular depolarizations, torsades and fibrillation. Progressive changes occurred over the duration of the epileptic syndrome. Interictal QT interval, measured for 5-s epochs where heartrate was 350 per min, progressively lengthened from a baseline of ~60 ms, by ~15 ms after >50 seizures. Prolonged QT most likely reflects changes in cardiac ion channels and may increase the risk of arrhythmia. Respiration was affected by epileptiform activity, including interruptions of respiratory rhythm after ECoG spikes and hyperventilation >120 per min during postictal immobility. These seizure-related cardiac and respiratory dysfunctions should be considered as potential risk factors for SUDEP.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: UIUC/College of Medicine Startup Funds (CAC)

Title: Elevated gonadotropin-releasing hormone neuron firing activity in a female mouse model of temporal lobe epilepsy

Authors: J. LI[1], V. A. ABEJUELA[2], J. KIM[2], J. B. LAMANO[2], N. J. KLEIN[2], M. A. GHANE[2], D. J. REYNISH[2], *C. A. CHRISTIAN[3,1,4,2];
[1]Neurosci. Program, [3]Mol. and Integrative Physiol., [4]Col. of Med., [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Reproductive dysfunction is a major co-morbidity of temporal lobe epilepsy for both men and women. A large degree of reproductive dysfunction appears to arise from seizure activity, but the mechanisms linking temporal lobe seizures to the control of reproduction are unclear. In order to assess the neural control of reproduction in the epileptic brain, we used a mouse model of temporal lobe epilepsy in which the convulsant kainic acid (KA) is stereotaxically injected into the hippocampal CA1 region. Control mice were injected with saline vehicle. First, we assessed the reproductive function of female C57BL/6J mice by daily vaginal smears for up to 2 months after KA/saline injection (saline n=10 mice; KA n=12). The majority of KA-treated mice developed disrupted estrous cycles within 2 months after injection. The estrous cycle irregularity was characterized by increased time spent in diestrus (saline 38.01%; KA 50.77%; p=0.04) and longer cycle length (saline 5.00 ± 0.15 d; KA 9.54 ± 1.62 d; p=0.03). We hypothesized that this disruption to estrous cyclicity arises, at least in part, via disrupted function of the hypothalamic gonadotropin-releasing hormone (GnRH) neurons, which form the final common pathway in the central control of reproduction. Therefore, we applied this epilepsy model to mice expressing the red fluorescent protein tdTomato in GnRH neurons. The spontaneous firing activity of GnRH neurons was recorded in acute brain slices by targeted extracellular loose patch recordings at 2 months after injection. GnRH neurons from KA-treated mice with irregular cycles showed a significantly higher average firing rate than control (p= 0.03) (saline 0.31 $\pm$ 0.06 Hz n=19 cells from 9 mice; KA/irregular 2.07 $\pm$ 1.11 Hz n=18 cells from 7 mice). Importantly, the mean firing rate of GnRH neurons from KA-treated mice that maintained regular cycles was not changed (KA/regular n=11 cells from 5 mice; p>0.1 compared to saline control). This suggests that the elevated GnRH neuron activity and estrous cycle disruption may be correlated. In male mice, by contrast, KA treatment did not significantly affect GnRH neuron firing rate (saline n=12 cells from 8 mice; KA n=15 cells from 8 mice; p>0.5),

indicating a potential sex difference in the response of the GnRH network to intrahippocampal KA injection. This is the first direct demonstration of GnRH neuron dysfunction associated with epilepsy. In summary, the intrahippocampal kainic acid mouse model of temporal lobe epilepsy appears appropriate for studying epilepsy-associated female reproductive dysfunction, which may be correlated with elevated GnRH neuron activity.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: Department of Biotechnology, Ministry of Science & technology, Govt. of India [Grant: BT/01/COE/09/08]

Title: Resting state glutamatergic activity revealed two independent epileptogenic networks in mesial temporal lobe epilepsy

Authors: ***J. BANERJEE**[1], A. DIXIT[1], V. VISHWANATHAN[1,2], A. SRIVASTAVA[3], B. RAMANUJAM[4], M. TRIPATHI[4], P. SARAT CHANDRA[3];
[1]Natl. Brain Res. Ctr., Gurgaon, India; [3]Neurosurg., [4]Neurol., [2]All India Inst. of Med. Sci., New Delhi, India

Abstract: The most common form of drug-resistant epilepsy is mesial temporal lobe epilepsy (MTLE), where the temporal lobe structures are involved in seizure generation. MTLE is associated with aberrant networks even under resting state, suggestive of abnormal synaptic connections. Excessive glutamatergic synaptic activity is one of the mechanisms associated with epileptiform activity in patients with MTLE but it is not clear if the magnitude of alteration in glutamatergic tone remain homogenous throughout the temporal lobe. Measuring the excitatory transmission under resting state may provide valuable information about region specific functional networks. In this study we have performed resting state coherence analysis of magnetoencephalography data of patients with MTLE who were undergoing epilepsy surgery. This revealed two major epileptogenic networks, one emanating from the hippocampus and the other from the anterior temporal region (ATL). Whole cell patch-clamp technique was used to record spontaneous excitatory postsynaptic currents (EPSCs) under resting conditions from pyramidal neurons in slice preparations of resected samples from the hippocampal and ATL

region obtained from patients with MTLE. We observed a higher frequency and amplitude of EPSCs in both the samples compared to non-epileptic controls, while the frequency was significantly high in ATL samples even in comparison to the hippocampal samples. To isolate action potential (AP)-dependent EPSCs from AP-independent EPSCs we performed the recordings in the presence of Na^+ channel blocker, tetrodotoxin (TTX; 200 nM). TTX reduced the frequency of spontaneous EPSCs by 49.6 ± 4.3 % and 61.8 ± 6.2 % in the hippocampal and ATL samples, respectively, suggesting that the magnitude of contribution of AP-dependent excitatory activity is higher in the ATL region as compared to the hippocampus. Compared to non-epileptic controls, peak amplitude of miniature EPSCs was higher by 47.8 ± 4.3 % in the hippocampus and by 64.8 ± 5.4 % in the ATL samples indicating difference in the glutamate release probability in the two regions. We also found that the magnitude of upregulation of expression of NR2A subunit of the NMDA receptors was more in the ATL samples compared to the hippocampal samples. These findings altogether support the concept that mechanism of hyper excitability varies in the hippocampal and the ATL region of patients with MTLE, possibly mediated by two independent epileptogenic networks.**Support**: This work is supported by Department of Biotechnology, Ministry of Science & Technology, Government of India [Grant: BT/01/COE/09/08].

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 406.08/J9

Topic: B.11. Epilepsy

Title: Modulation of thalamic and cortical GABA transporter: the potential mechanism for the anti-absence activity of mGlu5 receptors.

Authors: R. CELLI[1], I. SANTOLINI[1], V. D'AMORE[1], A. PITTALUGA[2], *R. GRADINI[3,1], G. VAN LUIJTELAAR[4], G. BATTAGLIA[1], V. BRUNO[1,3], F. NICOLETTI[1,3], R. T. NGOMBA[5]; [1]I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Dept. of Pharm., Univ. of Genova, Genova, Italy; [3]Univ. Sapienza, Roma, Italy; [4]Biol. Psychology, Donders Ctr. for Cognition, Radboud Univ., Nijmegen, Netherlands; [5]Univ. of Lincoln, Lincoln, United Kingdom

Abstract: Recent evidence suggests a protective role for Group I metabotropic glutamate receptors as potential candidates for the treatment of absence epilepsy. It has been demonstrated that acute treatment with positive allosteric modulators (PAMs) of mGlu1 and mGlu5

metabotropic glutamate receptors (RO0711401 and VU0360172, respectively) dose dependently reduces the incidence of spike-and wave discharges (SWDs), which are the EEG hallmarks of absence epilepsy, in spontaneously epileptic WAG/Rij rats. In addition, VU0360172 maintained its activity during chronic treatment, whereas rats developed tolerance to RO0711401 since the 3rd day of treatment and were still refractory to the drug two days after treatment withdrawal. VU0360172 was able to significantly reduce the incidence of SWDs when locally infused into the somatosensory cortex or the ventrobasal thalamus of WAG/Rij rats, and, interestingly, the action of intrathalamic VU0360172 was reversed by GABA transporter (GAT-1) inhibitor, tiagabine, infused in the thalamus at doses that did not affect SWDs on their own. On the basis of these findings, we have hypothesized that mGlu5 receptors regulate the expression and/or activity of GAT-1, and this specific mechanism might contribute to the role played by these receptors in the regulation of SWDs. We report here that a single injection of VU0360172 (3 mg/kg, s.c.) enhanced GAT-1 protein levels in the thalamus of symptomatic WAG/Rij rats (5 months of age), but did not change GAT-1 expression in the cerebral cortex or hippocampus. In contrast, no changes in thalamic GAT-1 expression were observed after 3 days of treatment with VU0360172 (3 mg/kg, s.c., bid), suggesting the development of tolerance. Curiously, a 3-day treatment with VU0360172 enhanced GAT-1 expression in the cortex and hippocampus. Identical findings were obtained in pre-symptomatic WAG/Rij rats (20-24 days of age) and in age-matched non-epileptic Wistar control rats, suggesting that the regulation of GAT-1 by mGlu5 receptors is not restricted to epileptic animals. Our data raise the possibility that activation of mGlu5 receptors controls the incidence of absence seizures by enhancing GABA uptake in the cortico-thalamo-cortical circuitry, thereby restraining the endogenous activation of $GABA_A$ and $GABA_B$ receptors.

Disclosures: **R. Celli:** None. **I. Santolini:** None. **V. D'Amore:** None. **A. Pittaluga:** None. **R. Gradini:** None. **G. van Luijtelaar:** None. **G. Battaglia:** None. **V. Bruno:** None. **F. Nicoletti:** None. **R.T. Ngomba:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Program#/Poster#: 406.09/J10

Topic: B.11. Epilepsy

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CARIPLO Foundation Grant 2013 0879

Title: A novel fluorescent probe for monitoring extracellular pH shifts during neuronal hyperactivity

Authors: *M. CHIACCHIARETTA[1], S. LATIFI[1], M. FADDA[2], M. BRAMINI[1], A. FASSIO[2], F. CESCA[1], F. BENFENATI[1];
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Abstract: In the central nervous system changes in extracellular pH modulate neuronal excitability, membrane potential, synaptic transmission and neurotransmitter uptake. The correlation between pH shifts and neuronal hyperexcitability is well established, however, because of the lack of accurate systems able to measure extracellular H^+ concentration, addressing the detailed events underlying pH-induced modulation of neural excitability is still a major challenge. Monitoring these pH shifts with high time and spatial resolution could help to elucidate many physiological and pathological processes. The aim of the present study is to describe the i) spatio-temporal dynamics of extracellular pH during neuronal hyperexcitability, and ii) the mechanisms underlying this process. To address these issues we created ex.E^2GFP, a membrane-targeted extracellular ratiometric pH indicator exquisitely sensitive to acidic shifts. By monitoring E^2GFP fluorescence in real time in primary cortical neurons we were able to build a dose-response curve by switching the excitation wavelength between 405 and 488 nm and systematically adjusting the extracellular pH from 5.9 to 7.7. We, then, quantified pH fluctuations during network excitation induced by bicuculline or by high frequency stimulation. Our data demonstrated that during epileptic-like activity pH shifts take place both at synaptic regions and at the soma of neuronal cells, both at excitatory and inhibitory sites. These acidic shifts during intense neuronal activity imply non-quantal release of protons from neurons; this process could occur through distinct H^+ secreting/transporting mechanisms, including the Na^+/H^+ exchanger (NHE), which transports Na^+ into the cells and extrudes H^+. Interestingly inhibition of NHE by amiloride blocks bicuculline-induced extracellular acidification, without affecting the firing and bursting rates, thus highlighting a major role of NHE in the regulation of extracellular pH. In conclusion, the extracellular sensor engineered in this study will be instrumental to better understand ionic dynamics under physiological and pathological conditions with high spatio-temporal precision and accuracy.

Disclosures: **M. Chiacchiaretta:** None. **S. Latifi:** None. **M. fadda:** None. **M. bramini:** None. **A. Fassio:** None. **F. Cesca:** None. **F. Benfenati:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 406.10/J11

Topic: B.11. Epilepsy

Support: NSC 102-2628-B-010-008-MY3

V103E9-003

V104C-068

Title: Involvement of toll-like receptor 2 in inflammatory responses and seizure generation in two mouse models of temporal lobe epilepsy

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Abstract: Objective: Neuroinflammation identified in epileptogenic tissue has been proposed to play central pathogenic roles in the development of neuronal cell death, reactive gliosis, aberrant neurogenesis and synaptic reorganization following epilepsy. Microglial cells are the principle immune cells in the innate immune system of central nervous system and their activation via toll-like receptor 2 (TLR2) has a pivotal role in pro-inflammatory responses associated with brain injury and some neurological diseases. Thus, it is hypothesized that seizure activities up-regulate TLR2 expression that might subsequently contribute to epileptogenesis. In the present study, we investigated the involvement of TLR2 in the development of epilepsy using two mouse models of temporal lobe epilepsy. **Methods:** Experimental epileptic seizures were induced by either pilocarpine or electrical amygdala kindling in both wild-type (WT) C57BL/6 mice and TLR2 knockout (TLR2 KO) mice. The TLR2 expression in the hippocampus was detected by immunohistochemistry and enzyme-linked immunosorbent assay. The effects of TLR2 inhibited neutralizing antibody, and of the genetic deletion of TLR2 on the development of epilepsy were evaluated. **Results:** In the epileptic hippocampus of WT mice, TLR2 was mainly expressed in microglia (Iba-1^{+}) in the regions of CA1, CA3 and dentate gyrus. Expression of TLR2 was significantly increased on day 7 after pilocarpine-induced status epilepticus. In the kindling model, the number of stimulations required to achieve fully kindled seizures was significantly decreased in the TLR2 KO mice compared to the WT mice. In addition, the TLR2 KO mice exhibited a shorter latency for seizure onset and higher after-discharge amplitude after electrical stimulation. Moreover, a reduced number of stimulation required to achieve fully kindled seizures was significantly decreased in the mice treated with TLR2 neutralizing antibody 30 min

prior to electrical kindling. In fully kindled mice, the relative threshold for seizure-induction showed a decreasing trend after TLR2 neutralizing antibody administration. **Conclusions:** Taken together, we assumed that TLR2 inhibition might promote kindling epileptogenesis and suggested that TLR2 participates in the generation of epilepsy.

Disclosures: Y. Hung: None. **Y. Tseng:** None. **Y. Wu:** None. **Y. Lin:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: B.11. Epilepsy

Support: MCST R&I-2013-14

Title: Role for 5-HT2C receptors in absence seizures: an electrophysiological and immunohistochemical study in GAERS and NEC rats

Authors: *G. DI GIOVANNI[1], M. VENZI[2], A. CAVACCINI[3], C. BOMBARDI[4], V. CRUNELLI[5];
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Abstract: Monotherapy with first-line anti-absence drugs only controls absence seizures (ASs) in 50% of patients, and there is therefore the need of novel therapeutic targets. In this study, we investigated the effect of selective 5-HT_{2C}R ligands on ASs in freely moving GAERS, a polygenic model of AS, and the modulation of tonic GABA-A current of ventral basal (VB) thalamocortical (TC) neurons as well as compared 5-HT_{2C}R and 5-HT transporter (SERT) expression in VB and perioral region of primary somatosensory cortex (PoS1) of GAERS and (non epileptic control) NEC strains. The 5-HT_{2C} agonist RO60-0175 suppressed ASs and normalized aberrant tonic current in GAERS VB TC neurons, an effect blocked by the selective 5-HT_{2C}R antagonist SB242084. Significant down-regulation of 5-HT_{2C}R-immunoreactivity (IR), but no difference in SERT-IR, was observed in VB and PoS1 of GAERS compared to NEC. In conclusion, 5-HT_{2C}Rs negatively control the expression of ASs in GAERS and decrease VB tonic GABA-A inhibition. Thus, dysregulation of 5-HT_{2C}Rs may be involved in the pathogenesis of absence seizures or a consequence of ASs. Crucially, selective 5-HT_{2C}R agonists might be potential novel anti-absence drugs.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: LGS Foundation

NIH NINDS R00 NS085046

NIH / NINDS T32 NS045540

Title: Role of Chd2 in cortical development and function

Authors: ***S. ABBASI**, J. C. FRANKOWSKI, S. LEE, K. GONZALEZ, S. SMITH, R. F. HUNT;
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Abstract: *De novo* mutations (or deletions) in the chromatin remodeler, Chd2, have been linked to a number of childhood brain disorders, such as epilepsy, intellectual disability, autism and/or photosensitivity. While increasing evidence supports a role of Chd2 in these disorders, how Chd2 mutations alter the function of individual subpopulations of neurons and ultimately brain circuits is unknown. To address the role of Chd2 deletion in cortical development, we generated a new floxed Chd2 transgenic mouse and crossed these animals to β-actin-Cre mice to produce Chd2+/- mutants. At P30, Chd2 was highly expressed in neurons (NeuN), including GABAergic interneurons (GAD67), but absent in putative astrocytes (GFAP) of wild-type animals. Western blot analysis showed a marked reduction of Chd2 protein in brain of Chd2 mutants. Patch-clamp recordings revealed an increase in excitatory synaptic input onto layer II/III pyramidal cells in somatosensory cortex of Chd2 mutants; intrinsic electrophysiological properties were unchanged. In addition, using a battery of behavioral tests, long-term video-EEG monitoring and immunostaining experiments, we are investigating the effect of Chd2 deletion in different cell types and at defined stages of forebrain development. Our results suggest cell type-specific impairments in cortical development and function may contribute to behavioral disturbances associated with Chd2 mutation.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant 2R01NS060757-05A1

Title: Neural activity propagation by endogenous electric field in the hippocampus *In vitro*

Authors: *R. SHIVACHARAN, D. DURAND;
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Abstract: It has been observed that epileptogenic agents generate fast-propagating waves that travel along the longitudinal pathway in the hippocampus. Recent studies in human epilepsy have described a similar phenomenon. We have previously shown that the fast-propagating wave travels at the speed of 0.12 m/s in the rodent hippocampi, which is not consistent with diffusion. In addition, these waves propagate in the absence of synaptic transmission and gap junction. These results indicate that the propagation mechanism is consistent with electric field transmission but do not provide direct evidence. The purpose of this study is to test the hypothesis that epileptiform activity propagates via electric field. We developed an extracellular field clamp (EFC) capable of measuring the local field and applying a current to cancel it. The experiments were carried out in a longitudinal hippocampal slice preparation with 4 Aminopyridine (4-AP) to generate spontaneous spikes observed to travel from the temporal to the septal region. Low-calcium solutions were applied to eliminate synaptic transmission. The EFC was located in the middle of the slice and the results show that spike could travel to the EFC but could not cross. This result supports the notion that electric field alone can be responsible for volume conduction of neural activity and that this haptic propagation could play a role in synchronization of epileptiform activity.

Disclosures: R. Shivacharan: None. **D. Durand:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: This work is supported by a collaborative FAU Seed Grant awarded to Drs. Isgor & Guthrie.

Title: Emergence of epilepsy in a transgenic mouse strain that overexpresses brain-derived neurotrophic factor in the forebrain

Authors: C. YEPES[1], M. LAQUERRE[1], W. ZHOU[1], K. GUTHRIE[1], *C. ISGOR[2];
[1]Biomed. Sci., [2]Charles E. Schmidt Biomed Ctrr, Florida Atlantic Univ. Charles E Schmidt Col. of Med., Boca Raton, FL

Abstract: Mice that overexpress brain-derived neurotrophic factor (BDNF) under the alpha-calcium/calmodulin-dependent protein kinase IIa promoter (termed TgBDNF mice) develop spontaneous seizures at ~6 months of age. Slow developing nature of behavioral disruptions observed in TgBDNF mice led to the hypothesis that chronic and sustained elevations in local BDNF are critical for progressive remodeling of hippocampal circuits implicated in epileptogenesis. We have previously shown that the mossy fibres (MF), axonal projections from the dentate gyrus granule cells (GCs) that innervate the CA3 field, are expanded in volume in TgBDNF mice compared to wildtype (WT) controls at 2-3 months of age coupled with increases in the molecular layer volume and dentate GC dendritic spine density. Increase in number of spines was observable in mushroom-type spines, suggesting that excess BDNF may be associated with increased synaptic input from entorhinal cortex onto GC dendritic arbors in mature circuitry even before seizure development. In this study, we monitored emergence of spontaneous seizures in the TgBDNF mice from 2 to 13 months of age to determine when seizures emerge and how severe and prevalent they become to assess the progressive nature of pathology. We also obtained electroencephalogram (EEG) recordings to demonstrate seizure profiles in cortical activity of epileptic mice. Lastly we tested for shifts in the balance of excitatory/inhibitory synaptic inputs to dentate GCs and CA3 neurons to determine emergence of hyperexcitable circuitry in the pre-seizure time period. Confocal imaging of presynaptic markers for excitatory or inhibitory synaptic proteins (VGLUT1, VGAT) are used to quantify densities of immunoreactive (IR) puncta in the molecular layer, stratum lucidum, stratum oriens, as well as granule and CA3 pyramidal cell layers. Puncta analyses are compared to those from 6-7 month-old TgBDNF mice that manifested seizures. 44% of TgBDNF mice displayed spontaneous seizures at ~6 months of age. This number increased to 85% of mice when animals mature to 12-13 months of age. The frequency and severity of seizures also increased with age, underlying the

progressive nature of epileptogenic processes. Epileptic seizures were marked with pre-ictal single spike events followed by ictal discharge, ending with EEG flattening that is associated with muscle stiffening (tonus) and loss of posture. The entire stage 5 seizure from pre-ictal spike events to background EEG recovery lasted about 30-60 sec. Animals recovered fully without any fatality. Our findings identify a progressive structure-function relationship with end point of epileptogenesis in the TgBDNF strain.

Disclosures: **C. Yepes:** None. **M. Laquerre:** None. **W. Zhou:** None. **K. Guthrie:** None. **C. Isgor:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant NS090041

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Title: Selective activation of somatostatin or parvalbumin expressing interneurons triggers GABA-mediated LLDs in rat neocortex

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Abstract: In the presence of the A-type K^+ channel blocker 4-aminopyridine (4-AP), long-lasting depolarizations (LLDs) occur spontaneously and propagate through the neocortex. These LLDs persist when excitatory glutamatergic neurotransmission is blocked with CNQX and D-APV (EAA blockers), suggesting the events arise from synchronous activity of inhibitory interneurons and represent a propagating GABA-mediated LLD. While this phenomenon is well documented, the role of specific interneuron (IN) classes in generating these events is poorly understood. Recently, we have shown that 4-AP alters the action potential and repetitive firing properties of Martinotti cells (MC) and fast-spiking basket cells (FS-BC) in neocortex, making these cells prime candidates for involvement in LLD initiation. In this study, we independently assessed the ability of MCs and FS-BCs to initiate LLDs using light-activation via genetically encoded channelrhodopsin (ChR) driven by the somatostatin (SST) or parvalbumin (PV) promoter, respectively. Spontaneous and light-initiated LLDs were recorded from putative Layer

2/3 MC, FS-BC and pyramidal cells in normal saline and following application of 4-AP and EAA blockers. In all cell types, wash-on of 4-AP and EAA blockers induced spontaneous LLDs and increased the amplitude and duration of light-triggered GABAergic events. The amplitude, duration, and response area of light-initiated LLDs in both PV:ChR and SST:ChR animals were statistically equivalent to spontaneous and evoked LLDs, suggesting activation of either interneuron class alone is sufficient to initiate cortical GABA LLDs. These results suggest synchronous GABAergic network activity is driven by neuronal ensembles consisting of multiple IN populations rather than being driven by a single cell type, implicating inter-population cooperativity in aberrant GABAergic activity in the neocortex.

Disclosures: **A. Bohannon:** None. **J. Hablitz:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: Medical Research Council

University of Edinburgh CMVM studentship

Title: Defective synaptic vesicle recycling in epilepsy and developmental delay-associated de novo mutations of dynamin 1

Authors: *K. BONNYCASTLE[1], D. C. SOARES[2], W. W. K. LAM[2], M. A. COUSIN[1]; [1]Muir Maxwell Epilepsy Ctr. and Ctr. for Integrative Physiol., [2]Inst. of Genet. and Mol. Medicine, Western Gen. Hosp., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The large neuron-specific GTPase, dynamin 1, plays an essential role in synaptic vesicle (SV) recycling. During clathrin-mediated endocytosis (CME), dynamin 1 induces fission of the membrane through guanosine-5'-triphosphate (GTP) hydrolysis at the vesicle neck. Dynamin 1 also plays a role in the formation of vesicles from bulk endosomes, following activity-dependent bulk endocytosis (ADBE). We have identified *de novo* dynamin 1 mutations in patients with either epilepsy, developmental delay, or comorbid for both of these disorders. We hypothesized that these *de novo* mutations in dynamin 1 may alter SV endocytosis. We evaluated the impact of these mutations on the 3-D structure of dynamin 1. All mutations identified were predicted to be deleterious for dynamin 1 function. To determine whether these mutations impacted on the synaptic role of dynamin 1, we used the genetically encoded

reporters, synaptophysin-pHluorin (sypHy) and vesicle associated membrane protein 4-pHluorin (VAMP4-pHluorin) to visualize both CME and ADBE in embryonic mouse hippocampal neurons. Intriguingly, we identified that specific dynamin 1 mutants had differential effects on both CME and ADBE. These defects in SV endocytosis may therefore underlie some of the symptoms displayed by these patients. They also suggest that dysfunctional SV endocytosis may be a common underlying event across different neurodevelopmental disorders, providing a novel avenue for future treatment strategies. Finally, these studies provide further insight regarding the potentially divergent molecular role(s) of dynamin 1 in presynaptic function.

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Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant R37/RO1-NS-3543

Title: Selective inhibition of inflammatory cascades following experimental febrile status epilepticus

Authors: *M. M. CURRAN, K. P. PATTERSON, M. SARGIOUS, T. Z. BARAM;
Univ. of California Irvine, Irvine, CA

Abstract: Rationale: Epilepsy is a common neurological disorder, effecting ~1% of the world's population. Temporal lobe epilepsy (TLE) is a common form of epilepsy, and is often preceded by trauma or long febrile seizures (febrile status epilepticus, FSE) (French et al., 1993; Dubé et al., 2007). However, how the insult, including FSE, promotes epilepsy is unknown. Inflammation is involved in fever and FSE and is among candidate mechanisms for epileptogenesis (Vezzani, et al., 2011). Using a model of experimental FSE, we have previously shown that FSE is sufficient to directly cause TLE in a subset of immature rodents (Dube, et al., 2006, 2010) and have found a strong correlation of inflammatory cytokine expression and epilepsy-predicting limbic MRI changes (Patterson, et al., 2015). This prompted the hypothesis that early, selective interference with initial steps of inflammatory pathways would prevent later-phase inflammatory mediators and abort the epileptogenic processes. Because global elimination of inflammation was not helpful (Patterson, et al., in prep), we focused on High Mobility Group Box 1 (HMGB1) and EP2 prostaglandin E_2 (PGE_2) receptor as potential therapeutic targets.

Methods: Experimental FSE was induced in post-natal day 10-11 Sprague Dawley rat pups as previously described (Choy, et al, 2014). Briefly, pups were exposed to a continuous stream of warm air to induce behavioral seizures. Elevated core temperatures and seizures were maintained for 60 minutes. In the first experiment, FSE or normothermic control rats were given intracerebroventricular infusions of vehicle or HMGB1 Box A, a direct antagonist of HMGB1 (Maroso, et al., 2010). In the second experiment, rats were either given intraperitoneal injections of vehicle or TG6-10-1, a specific EP2 (a PGE_2 receptor) antagonist (Jiang et al., 2013). In both experiments, experimental rats or same litter naïve controls were then sacrificed and their hippocampi were extracted. qRT-PCR was used to quantify expression changes in inflammatory mediators.
Results: At both early time points (3 hrs) and later (24-96 hrs), there was robust activation of both the HMGB1- and PGE_2-dependent inflammatory pathways. This included elevations of Tumor Necrosis Factor-Alpha (TNFα), Inhibitor of Kappa B-Alpha (IκBα), Cyclooxygenase 2 (COX2) and membrane bound PGE-synthase 1 (mPGES1). Blocking HMGB1 binding to target receptors had little effect on the expression of HMGB1 dependent molecules as compared to both controls and FSE-vehicle animals. Experiments employing TG6-10-1 are ongoing.

Disclosures: M.M. Curran: None. **K.P. Patterson:** None. **M. Sargious:** None. **T.Z. Baram:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Pediatric Epilepsy Research from the Epilepsy Foundation

UVa SOM Grant

Title: The role of gluk2-containing kainate receptors in acute hypoxic seizures in the neonatal mouse.

Authors: *S. A. ZANELLI[1], P. WAGLEY[2], D. GROSENBAUGH[2], J. KAPUR[2];
[1]Pediatrics, Univ. of Virginia, Charlottesvle, VA; [2]Neurol., Univ. of Virginia, Charlottesville, VA

Abstract: Background: Kainate receptors (KARs) are differentially expressed in the brain during development and peak in the late embryonic/early postnatal period. Recent evidence supports the role of KARs, specifically those containing GluK2, in the pathogenesis of epilepsy. However, how KARs activation following hypoxia regulates synaptic transmission and seizures in the immature brain is not known.
Objective: To test the hypothesis that activation of KARs by glutamate during hypoxia alters synaptic transmission leading to increased seizure susceptibility in the neonatal mouse and that GluK2-containing KARs mediate, at least in part, these effects.
Methods: The effects of KAR-blockade on synaptic transmission were studied in an *in vitro* asphyxia model (oxygen glucose deprivation [OGD]). Miniature excitatory post-synaptic currents (mEPSCs) and miniature inhibitory post-synaptic currents (mIPSCs) were measured in CA1 pyramidal neurons in P7-10 mice. KARs were pharmacologically isolated using DL-AP5 (NMDA receptor antagonist), GYKI53655 (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) antagonist), UBP310 (KAR antagonist) or CNQX to block both AMPARs/KARs. Seizure generation following acute hypoxia (4min30secx4 min) was then studied in wild-type, GluK2 $^{-/-}$ and mice pre-treated with UBP310 (5-75 mg/kg, 30 min prior).
Results: mEPSC frequency increased during both OGD and reoxygenation. In the presence of UBP310, the increase in mEPSC frequency was abolished and instead a decrease in mEPSC frequency occured (0.36±0.04 Hz at baseline vs. 0.23±0.02 Hz during reoxygenation; p=0.0005). Further, exposure to OGD in the presence of GYKI53655 resulted in decreased mIPSC frequency during reoxygenation (0.362±0.06 Hz at baseline vs. 0.285±0.06 Hz during reoxygenation; p=0.0003). This decreased mIPSC frequency was not observed in similar experiments performed in the presence of CNQX to block AMPARs and KARs. Gluk2$^{-/-}$ mice were less susceptible to acute hypoxia with seizures during reoxygenation in 33.3% of pups vs. 80% in controls ($p<0.05$). Similarly, mice treated with UBP310 experienced less post-hypoxic seizures in a dose dependent manner with highest efficacy observed with 75 mg/kg (80% vs. 42.8%, $p<0.001$).
Conclusions: These results suggest that activation of GluK2-containing KARs during hypoxia represent an important mechanism of increased excitability in the neonatal brain. Further, KAR blockade leads to enhanced glutamatergic synaptic transmission while decreasing inhibitory synaptic transmission in the CA1 region of the hippocampus, emphasizing the role of KARs in the pathophysiology of neonatal hypoxic seizures.

Disclosures: S.A. Zanelli: None. **P. Wagley:** None. **D. Grosenbaugh:** None. **J. Kapur:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Program#/Poster#: 406.19/K2

Topic: B.11. Epilepsy

Support: WT098352MA

R01 NS044133-14

Title: Different electrographic epileptic discharge types associated with absence epilepsy are interrelated in rat association cortex.

Authors: *S. HALL[1], M. A. WHITTINGTON[1], R. D. TRAUB[2];
[1]Univ. of York, York, United Kingdom; [2]IBM T.J. Watson Res. Ctr., Dept. of Physical Sci., Yorktown Heights, NY

Abstract: Introduction
Spike and wave-like discharges (SpW) are a feature of many absence-type epilepsies. They can present in many forms, with some SpW types being linked to pathology of the thalamocortical axis. Here we demonstrate the generation of, and interrelationship between varying forms of SpW discharges in the neocortex, using a combination of *in vitro* electrophysiology and biologically realistic computational modelling.
Results
Slow SpW discharges were generated on a background of sleep-associated delta rhythms by a reduction of tonic neuromodulatory excitation of peptidergic inhibitory interneurons, mediated by nACh and/or 5HT3A receptors. This caused an increase in amplitude and slowing of the delta rhythm (the 'wave' component) generated by deep layer pyramidal cells, and a switch from single spike to burst discharges in superficial layers (the 'spike' component). SpW discharges were transformed into polyspike and wave (polySpW) discharges via two methods. First, the altering of the bias from NPY-mediated to VIP-mediated superficial peptidergic inhibitory interneurons was enhanced by targeting the specific peptides involved. This led to rapid disinhibition of the superficial layers, further increasing the superficial pyramidal cell output and leading to the generation of polySpW events. Second, an increase in deep- to superficial synaptic excitation, achieved through theta-burst stimulation of the deep layers, also led to the generation of polySpW events. The two polySpW models were distinguishable by the inter-spike intervals on each event. Peptidergic disinhibition resulted in short-interval (91.7 +/- 3.0 ms) polyspike discharges whereas theta-burst stimulation of the deep- to superficial pathway led to long-interval polyspike discharges (129.3 +/- 3.4 ms). The subtle difference in interspike intervals was accompanied by dramatic differences in interlaminar interactions: Granger causality estimates indicated that long-interval polySpW discharges were initiated in deep layers, influencing the

spikes seen in superficial layers.The converse was true for the short-interval polySpW discharges.
Conclusions
These data suggest at least two possible mechanisms behind the generation of polySpW events; 1) selective loss of inhibition in superficial layers of neocortex and 2) an increase in deep neuronal excitation of superficial layers, through an enhancement of deep to superficial excitation. Both polySpW types grossly interfered with the interlaminar dynamics associated with normal delta rhythms.

Disclosures: S. Hall: None. **M.A. Whittington:** None. **R.D. Traub:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 406.20/K3

Topic: B.11. Epilepsy

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AFA Försäkring 100222

Kungliga Fysiografiska Sällskapet

Title: Collagen VI extracellular matrix protein modulates short-term plasticity in the hippocampus: Implications for epileptogenesis.

Authors: *T. RAMOS-MORENO[1,2], A. CIFRA[2], L. NIKITIDOU-LEDRI[3], S. H. CHRISTIANSEN[3], C. GØTZSCHE[3], M. CESCON[4], P. BONALDO[4], D. P. WOLDBYE[3], K. MERAB[2];
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Abstract: Extracellular matrix (**ECM**) molecules modulate several aspects of synaptic plasticity in the central nervous system (**CNS**) through multiple mechanisms that ultimately affect learning and memory. Collagen VI (**CVI**) belongs to the category of ECM protein and is primarily known for its bridging role in connective tissues. Here we report that the mRNA and protein levels of Collagen VI (**CVI**) are increased in the rat hippocampus at 4 weeks after the initial insult in post *status epilepticus* model of chronic epilepsy. In addition, we further explore whether CVI plays a

role in synaptic plasticity and if it can functionally affect neuronal networks in the hippocampus. Surprisingly, we detect an increased basal synaptic transmission after exposing rat hippocampal slices to CVI for 2 h and paired-pulse facilitation in Schaffer collateral-CA1 excitatory synapses after incubating hippocampal slices with CVI for 6 h, whilst CVI deficient mice exhibited paired-pulse depression, the opposite effect. These data suggest that CVI may decrease overall release probability in Schaffer collateral-CA1 synapses and, since CVI protein levels are augmented in post-*status epilepticus* animals, this could help in counteracting increased excitability in epileptic neuronal circuits.

Disclosures: **T. Ramos-Moreno:** None. **A. Cifra:** None. **L. Nikitidou-Ledri:** None. **S.H. Christiansen:** None. **C. Gøtzsche:** None. **M. Cescon:** None. **P. Bonaldo:** None. **D.P. Woldbye:** None. **K. Merab:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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VA BLR&D CDA-2 005-10S

Ellison Medical Foundation

NIH R01-NS080979

NIH T32-T32DK007680

NIH P30-NS061800

Title: Structure and function of sprouted mossy fiber synapses in epilepsy.

Authors: *W. HENDRICKS[1,2], A. L. BENSEN[1], G. L. WESTBROOK[1], E. SCHNELL[2,3]; [1]Vollum Inst., Portland, OR; [2]Anesthesiol. and Perioperative Med., Oregon Hlth. and Sci. Univ., Portland, OR; [3]Portland VA Med. Ctr., Portland, OR

Abstract: Temporal lobe epilepsy is a debilitating chronic condition that can develop after various neurological insults, including traumatic brain injury, stroke, and infection. The exact mechanism driving the development of epilepsy has remained elusive. In the hippocampal dentate gyrus, seizures drive pathologic retrograde sprouting of granule cell mossy fiber axons.

Although sprouting may contribute to epileptogenesis, the physiologic consequences of mossy fiber sprouting are unknown. The dentate gyrus is a locus for adult neurogenesis and adult-born granule cells might directly give rise to sprouted mossy fibers, though this is widely debated and unresolved. If sprouted mossy fibers do primarily arise from adult-born dentate granule cells, then neurogenesis might play a role in establishing these aberrant connections. Using the pilocarpine model of epilepsy and transgenic labeling of age-defined cohorts of granule cells in mice, we demonstrate that adult-born neurons contribute to sprouting and are more likely to develop sprouted mossy fibers than their neonatally-born counterparts. Using super-high resolution confocal microscopy, we demonstrate these synapses form large and highly complex synapses onto dendritic shafts and spines. Furthermore, using optogenetics to drive granule cell firing, we find that sprouted mossy fibers drive recurrent excitation. When activated, these pathological synapses are capable of triggering firing of granule cells and propagating reverberating excitation throughout the dentate gyrus. Taken together, these data suggest that adult-born neurons may play a significant role in epileptogenesis through their contribution to mossy fiber sprouting.

Disclosures: W. Hendricks: None. **A.L. Bensen:** None. **G.L. Westbrook:** None. **E. Schnell:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Program#/Poster#: 406.22/K5

Topic: B.11. Epilepsy

Support: CIHR Grant MOP123258

Title: Potential role of β-amyloid peptides in kainic acid-induced toxicity

Authors: *D. I. OURDEV, A. KODAM, M. MAULIK, Y. WANG, M. BANERJEE, S. KAR; Univ. of Alberta, Edmonton, AB, Canada

Abstract: Kainic acid (KA), a degradation-resistant analogue of the excitatory neurotransmitter glutamate, is known to trigger seizures in rat models which originate from the hippocampus and can spread to other limbic structures. This is associated by the subsequent loss of neurons, mossy fiber reorganization, and astrogliosis, pathologies which closely mimic those characteristic of human temporal lobe epilepsy (TLE). KA exerts its epileptogenic effects through the activation of kainate receptors (KA-Rs) which generate synchronized network-driven glutamatergic currents. Nevertheless, the underlying cellular mechanisms by which KA triggers

neurodegeneration remains unclear. A number of recent studies indicate that amyloid β (Aβ), the peptides critical for their involvement in Alzheimer's disease (AD) pathogenesis, may play a potential role in triggering seizures and the associated loss of neurons. These peptides are generated from the constitutively expressed amyloid precursor protein (APP), which is alternately processed by the non-amyloidogenic α-secretase or amyloidogenic β-secretase proteolytic pathways. However, very little is known regarding the involvement of Aβ peptides in KA-induced toxicity. To address this issue, we evaluated time-dependent alterations in the levels and cellular distribution of APP and its processing enzymes in the hippocampi of KA-treated rats. We report that KA triggers a significant increase in APP processing in proliferating astrocytes. Additionally, using rat primary hippocampal neuronal cultures, we are currently evaluating the significance of Aβ peptides in the degeneration of neurons. Our results reveal that increased endogenous levels of APP may have a role in triggering degeneration of neurons in animal models of TLE.

Disclosures: D.I. Ourdev: None. **A. Kodam:** None. **M. Maulik:** None. **Y. Wang:** None. **M. Banerjee:** None. **S. Kar:** None.

Poster

406. Epilepsy: Synaptic and Post-Seizure Mechanisms

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Program#/Poster#: 406.23/K6

Topic: B.11. Epilepsy

Support: NIH Grant NS087095

Title: Regulation of synaptic transmission by mTORC1 and mTORC2.

Authors: *M. MCCABE, C. BARROWS, M. C. WESTON;
Dept. of Neurolog. Sci., Univ. of Vermont, Burlington, VT

Abstract: The mammalian target of rapamycin (mTOR) signaling cascade regulates cellular growth, proliferation and protein synthesis. Hyperactivation of mTOR in the central nervous system of both humans and animal models can lead to cortical malformations, cognitive disabilities, autistic behavior and epilepsy. In addition, the mTOR pathway may be abnormally activated by acquired injuries that produce epilepsy and trigger downstream processes of epileptogenesis. Therefore, a more complete understanding of how the mTOR pathway regulates fundamental aspect of neuronal function may provide new targets for therapeutic intervention early on or even prior to the onset of neurological symptoms. Pten is a negative regulator of the PI3K-mTOR signaling pathway whose loss of function causes mTOR hyperactivation. Deletion

of Pten in subsets of neurons leads to macrocephaly, autism-like behavior and seizures. Previous studies of synaptic transmission after genetic or pharmacological manipulation of the mTOR signaling cascade, and Pten in particular, have reported alterations in synaptic transmission and plasticity. Specifically, Pten loss leads to enhanced synaptic currents in both glutamatergic and GABAergic neurons, and increases in both the number of synaptic vesicles in the readily releasable pool and the postsynaptic response to single vesicle fusion are underlying mechanisms. 72 hour treatment with rapamycin completely blocked these changes and caused an additional increase in the probability of synaptic vesicle fusion. The goal of the present research was to explore the role of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) in mediating these effects. We selectively impaired mTORC1 or mTORC2 in cultured hippocampal neurons by genetic deletion of Raptor or Rictor, association proteins necessary for the formation of mTORC1 and mTORC2 respectively. We observed a reduction in peak evoked EPSC (eEPSC) amplitude following Raptor or Rictor knockout, as well as a reduction in the size of the synaptic vesicle readily releasable pool (RRP) following Raptor knockout. When both Raptor and Pten were deleted, peak eEPSC amplitude and RRP size resembled wild-type cells. Taken together, these observations indicate that mTORC1 and 2 play dissociable roles in regulating synaptic transmission, and loss of mTORC1 alone can rescue the synaptic effects of Pten knockout.

Disclosures: **M. McCabe:** None. **C. Barrows:** None. **M.C. Weston:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 407.01/K7

Topic: B.11. Epilepsy

Title: Hippocampal hypermetabolism following kainic acid administration in awake and anesthetized rats: FDG-PET study

Authors: *T. BERDYYEVA, Y.-C. HSIEH, S. YUN, J. SHELTON, C. DUGOVIC, H. KOLB, A. SZARDENINGS;
Janssen, San Diego, CA

Abstract: The effectiveness of novel anti-epileptic drugs (AEDs) is often judged by their effectiveness in reducing the severity of behavioral and electroencephalographic (EEG) signs of seizures in animal models. However, behavioral and EEG assessment provide limited insight into the extent of the seizure-related pathological changes within the relevant brain structures. Therefore, the selection of medication is often biased toward agents reducing seizure symptoms

rather than alleviating the seizure-related brain pathologies. Here, we demonstrate the use of the non-invasive brain imaging technique - positron emission tomography (PET) - in conjunction with EEG and behavioral monitoring as a screening tool to select treatments that not only alleviate seizures, but also lessen seizure-related pathological changes within the brain.
We used PET imaging to measure seizure-induced changes in the standardized uptake values (SUV) of [F18] fludeoxyglucose (FDG) in rats that behaved freely for 30 minutes following kainic acid (KA, 12 mg/kg, i.v.) administration ("uptake in awake", n = 10, static scan for 30 minutes). The electrographic seizure activity was confirmed via EEG recordings, and the behavioral seizure scores were recorded every 2 minutes. We observed that KA administration reliably, consistently and significantly elevated FDG uptake in the hippocampus relative to control (SUV changed from 3.56 to 4.78, 33% increase, p =0.005, t-test). To investigate the time course of the pathological hippocampal hyperactivity following KA administration, we performed a dynamic scan (60 minutes; the data were binned in 10-minute intervals) in rats that were continuously anesthetized ("uptake in anesthetized", n = 4). Similarly to the "uptake in awake", KA – treated rats showed increased FDG uptake in hippocampus, with the difference becoming significant 35, 45 and 55 minutes post-KA administration ($p< 0.05$, 0.01, 0.001 respectively). Interestingly, a standard treatment to alleviate KA-induced seizures in rat (combination of Ketamine, 50 mg/kg, i.p. and Diazepam, 20 mg/kg, i.p.) did not alleviate hippocampal hyperactivity in either group of animals, despite reducing KA-induced seizure severity.
These results demonstrate the presence of pathological hippocampal hypermetabolism not only in awake rats, but also in rats that were continuously anesthetized. Integrating non-invasive PET imaging with traditional assessment of seizures could potentially increase translatability of the drug screening process, leading to novel therapeutics designed to target and abolish abnormal patterns of both electrical and metabolic activity.

Disclosures: **T. Berdyyeva:** A. Employment/Salary (full or part-time): Janssen LLC. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Janssen LLC. **Y. Hsieh:** A. Employment/Salary (full or part-time): Janssen. **S. Yun:** A. Employment/Salary (full or part-time): Janssen LLC. **J. Shelton:** A. Employment/Salary (full or part-time): Janssen LLC. **C. Dugovic:** A. Employment/Salary (full or part-time): Janssen. **H. Kolb:** A. Employment/Salary (full or part-time): Janssen. **A. Szardenings:** A. Employment/Salary (full or part-time): Janssen.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

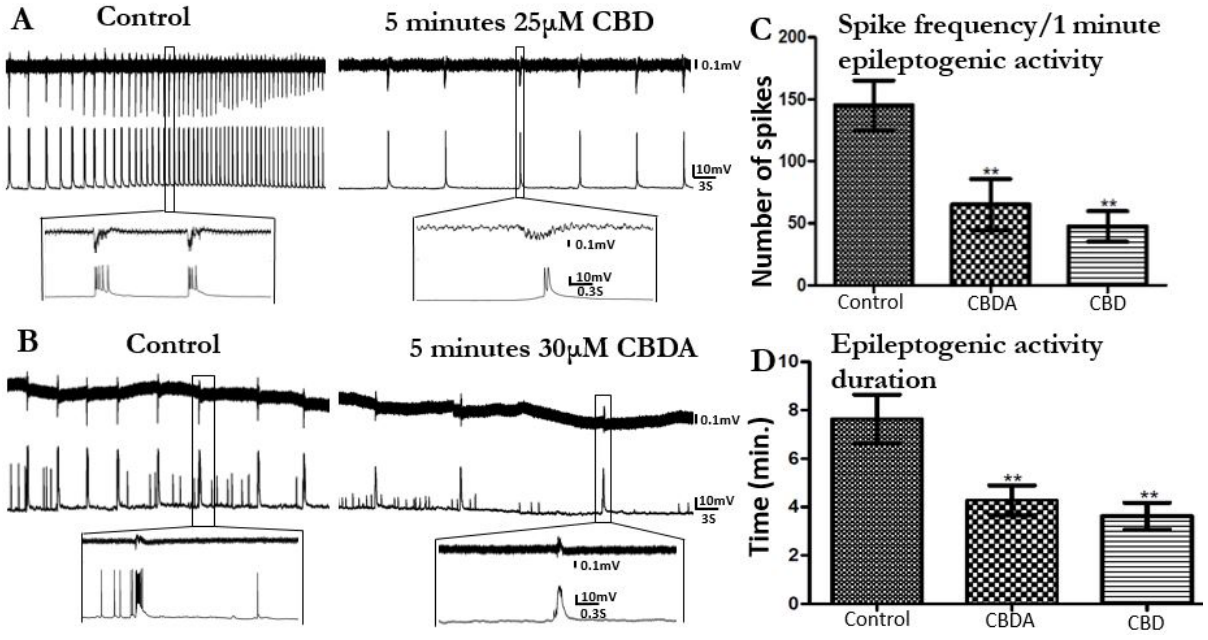
Program#/Poster#: 407.02/K8

Topic: B.11. Epilepsy

Title: Cannabidiolic acid controls seizure-like activity and neuronal excitability

Authors: *M. HOSSEINI ZARE[1], A. ABDULLA[1], K. AKULLA[2], J. ZIURKUS[1];
[1]Biol. and Biochem., Univ. of Houston, Houston, TX; [2]Ultragenyx Pharmaceut. INC., Novato, CA

Abstract: Intoxicating tetrahydrocannabinol (THC) and non-intoxicating cannabidiol (CBD) are the two most prevalent phytocannabinoids found in *cannabis sativa* plants. CBD is well studied in multiple disease models, especially as anti-convulsant in treatment of intractable forms of epilepsy. However, in many strains of cannabis, over 90% of phytocannabinoids are synthetized as non-intoxicating acidic cannabinoids. 'Raw hemp oil' extracts prepared using cold temperatures are often dominated by acidic cannabinoids, such as cannabidiolic acid (CBDA), which is the precursor of CBD. Recent evidence suggests that CBDA is an effective anti-emetic (acting through serotonin receptors), anti-inflammatory (Cox-2 inhibition), and it abrogates cancer cell growth. However, it is unknown what role CBDA plays in the central nervous system (CNS) and there is an urgent need to understand the functions of the acidic cannabinoids. Using single cell and network electrophysiological recordings, we compared effects of synthetic CBD (30μM) and CBDA (25μM) on seizure-like activity and neuronal excitability in the hippocampal slices. Both, CBD and CBDA reduced zero magnesium-induced seizure-like activity by reducing action potential firing in the excitatory pyramidal cells. CBDA actions were typically as effective, but delayed by 10-15 minutes, compared to CBD. Only CBDA significantly increased afterhyperpolarizations following the action potentials. In summary, our studies reveal new knowledge about the mechanisms of actions of CBDA in the brain. Further studies of CBDA and other acidic cannabinoids are needed to determine their effectiveness and therapeutic potential in vivo.



Disclosures: **M. Hosseini Zare:** None. **A. Abdulla:** None. **K. Akulla:** None. **J. Ziurkus:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Program#/Poster#: 407.03/K9

Topic: B.11. Epilepsy

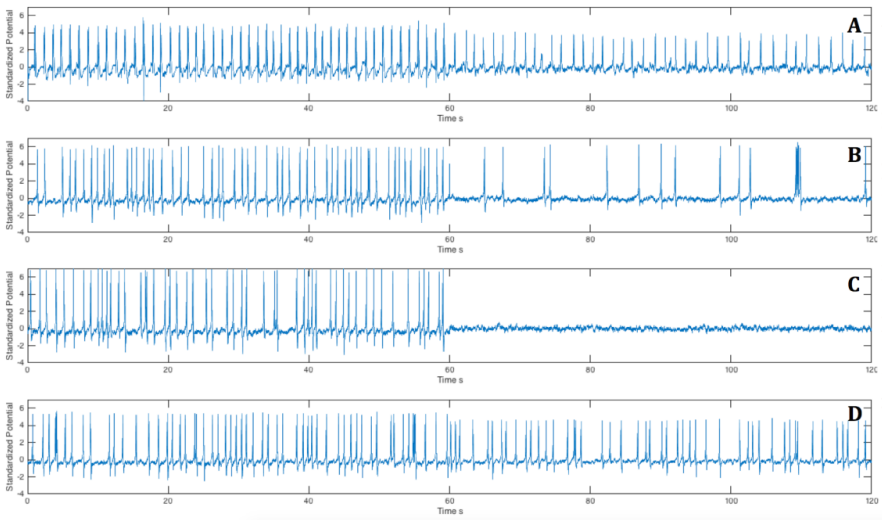
Support: Grant-in-Aid for Scientific Research(S) Grant Number 15H05719.

Title: A temperature-dependent neural mass model for suppression of epileptic discharges

Authors: ***J. SORIANO**[1,2], T. KUBO[1], T. INOUE[3], H. KIDA[3], T. YAMAKAWA[4], M. SUZUKI[3], K. IKEDA[1];
[1]Nara Inst. of Sci. and Technol., Nara, Japan; [2]Univ. of the Philippines - Diliman, Quezon City, Philippines; [3]Yamaguchi Univ., Ube, Japan; [4]Kumamoto Univ., Kumamoto, Japan

Abstract: An estimated fifty million people across the world currently live with epilepsy and suffer from seizures. Treatment for epilepsy ranges from anticonvulsants to invasive brain surgeries. Absolute remission however is not necessarily guaranteed. Focal brain cooling has been pursued and critically reviewed in the past decade as an alternative treatment for cases of refractory epilepsy. Previous research works with animal and human have suggested that cooling of the focal brain area significantly suppresses epileptic discharges. This observation is coincident with reduced concentration of neurotransmitters such as glutamate, which can be translated to reduced average synaptic gain of neural mass populations. In this study, we test whether this reduced concentration of neurotransmitters is a responsible mechanism for the effect of focal brain cooling via a temperature-dependent neural mass model using Q_{10} temperature coefficient. Wendling's model is used for its ability to reproduce multiple activity patterns observed in intracranial EEG only by changing average synaptic gains of dendritic and somatic inhibitory populations. Reduced neurotransmitter concentration is incorporated in the model using a Q_{10} factor in the conversion of input firing rate to average postsynaptic potential via a transfer function. We find that increasing Q_{10} factor values from 1.0 (no temperature dependence) decreases the frequency of epileptic discharges until complete termination. However, this does not capture persistent albeit suppressed epileptic discharges observed in EEG activities of drug-induced epilepsy in rats. This result suggests that a different mechanism is required to reproduce the observed effect of focal brain cooling. In additional analysis, we introduce a different Q_{10} factor in the conversion of input potential to average firing rate via a sigmoid function. Our results show that this model mimics suppression of epileptic discharges

during cooling as observed in the experiments implying that multiple responsible mechanisms may explain the effect of focal brain cooling.



A: EEG activity of induced epilepsy in rat brain before cooling (0s-60s) and during cooling (60s-120s) with T=20 ºC.
B: Model 1 with a Q_{10} factor of 1.006 in the transfer function. Frequency of epileptic discharges is reduced.
C: Model 1 with a Q_{10} factor of 1.013 in the transfer function. Epileptic discharges are completely terminated.
D: Model 2 with Q_{10} factors of 1.1 in the transfer function and 1.0985 in sigmoid function. This model replicates observed suppression of epileptic discharges.

Disclosures: **J. Soriano:** None. **T. Kubo:** None. **T. Inoue:** None. **H. Kida:** None. **T. Yamakawa:** None. **M. Suzuki:** None. **K. Ikeda:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Topic: B.11. Epilepsy

Support: NIH Grant NS025704

Citizens United for Research in Epilepsy

Title: Cannabidiol increases inhibitory transmission and rescues social deficits in a mouse model of Dravet Syndrome

Authors: ***J. KAPLAN**[1], W. A. CATTERALL[2], N. STELLA[2], R. E. WESTENBROEK[2]; [1]Dept. of Pharmacol., [2]Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Heterozygous mutations in the *Scn1a* gene encoding the brain type-1 voltage-gated sodium channel Nav1.1 cause Dravet Syndrome (DS), a severe childhood disorder characterized by treatment-resistant epilepsy, cognitive deficits, and autism spectrum disorder (ASD). As DS

symptoms result from the selective reduction of inhibitory interneuron excitability, therapeutic strategies that enhance interneuron excitability may effectively treat symptoms of DS. The phytocannabinoid, cannabidiol (CBD; GW Pharma; 10 mg/kg) represents a promising treatment for seizures that develop in DS, but its effects on ASD and cognitive impairment remain unknown. Here, we tested CBD's effects on ASD-like social deficits and on underlying physiological mechanisms in a mouse model of DS. *Scn1a*$^{+/-}$ mice (DS mice) phenocopy the core epilepsy, cognitive deficits, and ASD-like behavioral phenotypes associated with DS. CBD reduces seizure severity and duration in DS mice (Westenbroek et al., accompanying abstract), so we hypothesized that this compound may also reduce ASD behaviors in DS mice by restoring excitability to inhibitory interneurons. We tested this hypothesis using behavioral assays in which DS mice have well-characterized deficits, as well as patch-clamp electrophysiology of acutely dissected hippocampal slices in vitro. In the 3-Chamber Test, CBD improved the ratio of time DS mice spent in social interaction with a novel mouse compared to an empty cup. In the Reciprocal Interaction Test, CBD increased the number of social interactions and reduced defensive escape behaviors. These results suggest that CBD increased sociability and reduced social anxiety in DS mice. Next, we tested the effect of 16 μM CBD on excitatory and inhibitory input to DS mouse dentate gyrus granule cells. CBD increased the frequency of action potential (AP)-driven spontaneous inhibitory postsynaptic currents and reduced the frequency of spontaneous excitatory postsynaptic currents, without affecting amplitude. CBD reduced AP frequency in dentate granule cells in response to injection of depolarizing current below rheobase, but it had no effect on AP firing properties in response to depolarizing current above rheobase. Our results suggest that CBD rescues ASD-like behaviors and epileptic seizures by rebalancing the brain's excitatory/inhibitory ratio, primarily by enhancing inhibitory interneuron excitability, and thus represents an effective therapeutic strategy to rescue ASD behaviors in DS. Future experiments will test the effect of CBD on cognitive parameters and assess CBD's actions directly on interneuron firing properties.
Research supported by CURE (REW) and NINDS (WAC).

Disclosures: **J. Kaplan:** None. **W.A. Catterall:** None. **N. Stella:** None. **R.E. Westenbroek:** None.

Poster

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Topic: B.11. Epilepsy

Support: NIH Grant NS025704

Citizens United for Research in Epilepsy

Title: Cannabidiol reduces the duration and severity of thermally evoked seizures in a mouse model of Dravet Syndrome

Authors: *R. E. WESTENBROEK[1], J. S. KAPLAN[2], N. STELLA[2], W. A. CATTERALL[2]; [1]Pharmacol., Univ. Washington, Seattle, WA; [2]Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Dravet Syndrome (DS) is one of the most severe childhood neuropsychiatric diseases, with intractable epilepsy, febrile seizures developmental delay, ataxia, sleep disorder, autistic-like behaviors, frequent premature death and profound cognitive deficit. Clinical and preclinical results suggest that cannabidiol (CBD), a non-psychoactive ingredient produced by the *Cannabis* plant has antiepileptic effects that act through a novel mechanism of action. We tested the hypothesis that CBD reduces thermally evoked seizures in mice that have heterozygous loss-of-function mutations in the *Scn1a* gene encoding Nav1.1 voltage-gated sodium channels and have been shown to accurately phenocopy DS. DS mice were treated with increasing concentrations of CBD (GW Pharma) or vehicle one hour prior to raising their body temperature one-half of a degree every two minutes up to 38^{o}C (similar to a low fever) and then holding this temperature for 30 min. We found that CBD at 100 mg/kg and 200 mg/kg reduced the duration of thermally evoked seizures in DS mice but had no effect on the temperature dependence of evoked seizures. Additionally, CBD reduced the severity of thermally evoked seizures from a Racine score of 5.0 to less than 4.0. With this significant reduction in Racine score, DS mice are no longer at high risk for sudden unexpected death in epilepsy (SUDEP). Collectively, these results suggest that CBD may be an effective therapeutic agent for thermally evoked seizures associated with DS. The effects of CBD on social behavior inherent in the DS phenotype and on possible underlying mechanisms are reported by Kaplan et al. (accompanying abstract). Research supported by CURE (REW) and NINDS (WAC).

Disclosures: R.E. Westenbroek: None. **J.S. Kaplan:** None. **N. Stella:** None. **W.A. Catterall:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Program#/Poster#: 407.06/K12

Topic: B.11. Epilepsy

Support: NIH U01 NS074926-01

Title: Anticonvulsant, antiepileptic and neuroprotective effects of ketamine, valproate and midazolam polytherapy against soman exposure in rats

Authors: *L. A. LUMLEY[1], F. ROSSETTI[2], M. F. STONE[1], C. R. SCHULTZ[1], M. Q. PHAM[1], J. NIQUET[3], C. WASTERLAIN[3];
[1]USAMRICD, Gunpowder, MD; [2]Walter Reed Army Inst. of Res., Silver Spring, MD; [3]UCLA-West LA VAMC, Los Angeles, CA

Abstract: Introduction: Chemical warfare nerve agents (CWNA), such as soman (GD), produce *status epilepticus* (SE), resulting in extensive neuropathology and long-term performance deficits if seizures are not controlled. Current treatments for GD exposure increase survival, but are not fully protective especially when treatment is delayed. To identify a better treatment against pharmaco-resistant seizures caused by GD exposure, we are using combinations of drugs aimed at reversing the effects of maladaptive receptor trafficking that follows CWNA exposure. Methods: Rats were implanted with telemetry transmitters for continuous monitoring of EEG, body temperature and activity. After surgical recovery, rats were exposed to 1.2 LD_{50} GD and treated 1 min later with atropine sulfate and the oxime HI-6 and then 40 min after seizure onset with the histone deacetylase (HDAC) inhibitor valproate (VAL) with or without the NMDA antagonist ketamine (VAL/KET), the benzodiazepine midazolam (VAL/MDZ) or a combination of all three drugs (VAL/KET/MDZ). Two weeks after exposure, rat brains were sectioned and stained for neuropathology assessments. Results: Triple therapy with VAL/KET/MDZ reduced total time spent in seizures (SE + early recurrent seizures) in the first 72 h and reduced the number of spontaneous recurrent seizures compared to saline-treated or to those that received monotherapy or bi-therapy. Triple therapy prevented the development of hyperactivity that occurs in the weeks after GD exposure. In addition, triple therapy prevented loss of GABA interneurons in the piriform cortex and amygdala and loss of neurons in the piriform cortex, thalamus and amygdala that follows GD exposure. Conclusion: Triple therapy with VAL/KET/MDZ had remarkable anticonvulsant, antiepileptic, and neuroprotective effects compared with mono- or bi-therapy in GD-exposed rats. Triple therapy may be a highly effective approach against pharmacoresistant seizures, such as those caused by GD-exposure and may allow for use of lower doses with fewer side effects than those often seen with large doses of individual drug therapies. *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. The protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by the NIH to Dr. Claude Wasterlain.

Disclosures: **L.A. Lumley:** None. **F. Rossetti:** None. **M.F. Stone:** None. **C.R. Schultz:** None. **M.Q. Pham:** None. **J. Niquet:** None. **C. Wasterlain:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 407.07/K13

Topic: B.11. Epilepsy

Title: Retrospective analysis of acute seizure management of 215 pediatric cases with intravenous levetiracetam

Authors: *B. F. KIRMANI, ESQ[1,2], P. LAKIREDDY[3], M. DANG[4], A. SARODE[5], S. LONG[5], P. PATEL[5], O. KHAN[3];
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Abstract: Introduction: Children with intractable epilepsy have frequent hospital admissions because of status epilepticus and acute repetitive seizures. Seizures are also seen in the neonatal period and are a major indicator of future adverse neurological sequelae. Intravenous (IV) levetiracetam became available in August 2006 in patients aged 16 years and above. There is not enough data about safety and tolerability in children. We retrospectively analyzed data at our institution of children who received IV levetiracetam for acute seizure management. Objective: The goal of this study is to retrospectively assess the efficacy and tolerability of IV levetiracetam therapy in acute seizure management in children. Methods: A retrospective chart review was conducted on all preterm neonates, term neonates, and children less than 18 years of age who received IV levetiracetam at Scott and White Hospital/Texas A & M HSC College of Medicine, Temple, TX. Subject data were acquired from electronic medical records. Approval of this retrospective analysis was given by our hospital's Institutional Review Board. Results: We retrospectively analyzed 215 patients who met our inclusion criteria for neonatal seizures, status epilepticus and acute repetitive seizures and received IV levetiracetam. There were 119 (55.34%) males and 96 (44.65%) females. The loading dose of IV levetiracetam was 50 mg/kg in most patients, followed by a maintenance dose of 25 mg/kg every 12 hours. The dose was infused over 15 minutes to an hour. The primary objective was to assess response based on clinical and electro graphic documentation. The secondary objective was to assess the indication of initiation of this medicine, adverse events, and seizure control at well child visits. Response to levetiracetam was favorable. 185 (86%)out of 215 patients reached seizure freedom within 24 hours and 16 (7.44%) within 72 hours. Seizures continued after 72 hours in 14 patients requiring additional anticonvulsants. No serious side-effects were apparent. No serious adverse events were reported with any of the patients, 35 patients (20.7%) reported behavioral changes (irritability) while on levetiracetam. 14 patients were lost to follow-up . Patients were switched to oral levetiracetam after discharge from the hospital . The duration of follow up ranged from 6

months to 5 years. Conclusions: IV Levetiracetam seems to be efficacious and tolerable in acute seizure management in children.

Disclosures: B.F. Kirmani: None. **P. Lakireddy:** None. **M. Dang:** None. **A. Sarode:** None. **S. Long:** None. **P. Patel:** None. **O. Khan:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 407.08/K14

Topic: B.11. Epilepsy

Title: Ganaxolone and diazepam administered IV produce a synergistic anti-epileptic effect in a treatment refractory model of status epilepticus

Authors: *M. S. SAPORITO[1], J. A. GRUNER[2], J. TSAI[1], A. PATRONEVA[1];
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Abstract: Ganaxolone (GNX) is the 3β-methylated analog of the naturally occurring neurosteroid allopregnanolone that acts as a positive allosteric modulator of the $GABA_A$ receptor. GNX differs from the benzodiazepine class of $GABA_A$ modulators in that it enhances the activity of both the synaptic (comprised of α and γ subunits) and extrasynaptic $GABA_A$ receptors (comprised of α and δ subunits). The benzodiazepine sensitive synaptic $GABA_A$ receptor rapidly desensitizes during seizures and with repeated benzodiazepine administration. In contrast, the extrasynaptic $GABA_A$ receptor remains responsive to GNX during seizure events and does not desensitize with repeat GNX treatment. GNX blocks seizures in a broad range of experimental epilepsies and is now in clinical development for the treatment of various epileptic conditions. In recent preclinical studies we've demonstrated that IV administered ganaxolone blocked seizures in a clinically translatable model of benzodiazepine resistant status epilepticus (SE). The current studies were conducted to expand those findings by assessing the effects of the combination of IV administered GNX and diazepam on experimental SE. SE was induced by administration of lithium chloride and pilocarpine to Sprague-Dawley rats. Seizure response was measured by electroencephalographic recordings through pre-implanted cortical electrodes. Diazepam and GNX were administered alone or in combination via IV bolus administration 15 min after SE onset. The pilocarpine-induced seizures were sustained for approximately 6 hrs and were resistant to diazepam up to a dose-level of 10 mg/kg. In a previous study GNX at dose-levels of >12 mg/kg produced a complete and durable block of SE whereas lower doses were not protective. In the current study, diazepam at 5 mg/kg in combination with GNX administered at subtherapeutic dose-levels of 3 or 6 mg/kg caused a partial and complete block of SE,

respectively. The pharmacokinetics of GNX and diazepam were identical when administered alone or in combination indicating that neither drug affected the pharmacokinetic disposition of the other and suggesting that this synergistic enhancement of anti-epileptic activity occurred at the level of the $GABA_A$ receptor. Further, these data have clinical implications in the treatment of SE when GNX is to be administered to patients who are or have been treated with benzodiazepines.

Disclosures: **M.S. Saporito:** A. Employment/Salary (full or part-time): Marinus Pharmaceuticals, Inc. **J.A. Gruner:** 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; Melior Discovery, Inc. **J. Tsai:** A. Employment/Salary (full or part-time): Marinus Pharmaceuticals, Inc. **A. Patroneva:** A. Employment/Salary (full or part-time): Marinus Pharmaceuticals, Inc..

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Program#/Poster#: 407.09/K15

Topic: B.11. Epilepsy

Support: NIH 1U54 NS079202

Title: Using high-throughput screening to predict novel antiseizure interventions

Authors: ***A. MOUSAVI NIK**, S. HULSIZER, I. PESSAH;
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Abstract: Tetramethylenedisulfotetramine (TETS) is a potent convulsant that increases electrical activity and spontaneous Ca^{2+} oscillations in neural networks by blocking $GABA_A$ receptors. Acute exposure to TETS (≥ 3 μM) significantly increases spontaneous Ca^{2+} oscillations (SCO) amplitude and decreases SCO frequency. Since neurosteroids and benzodiazepines are positive allosteric modulators (PAMs) distinct mechanisms at $GABA_A$ receptors, we tested 27 neurosteroids and 2 benzodiazepines singly or in combination for their potency to mitigate TETS-triggered SCO abnormalities. SCOs were measured *in vitro* using mouse hippocampal neuronal cultures loaded with Flo-4 using FLIPR Tetra (Molecular Devices) high-throughput fluorescent cellular screening system. The SCOs were measured from mature networks at 12-15 days in vitro. Acute challenge to TETS rapidly altered the amplitude and frequency of SCOs and neurosteroids and benzodiazepines were tested for their ability to normalize SCO patterns. The

most potent candidates/combinations were also tested for their ability to normalize TES-triggered neuronal network electrical activity using multi-well micro electrode array (MEA, Axion) technology. Our results suggested that of 27 neurosteroids screened in hippocampal neuronal cultures, allopregnanolone, ganaxolone and XJ-42 are the most potent, and more potent than the benzodiazepines midazolam and diazepam. The combination of neurosteroids and benzodiazepines at low concentrations significantly reversed the effect of TETS and proved synergistic. High throughput screening using FLIPR and MEA to monitor SCO patters and Ca^{2+} dynamics is a potentially useful approach for identifying novel therapeutic agents that mitigate hallmarks of seizurogenic activity in vitro.

Disclosures: **A. Mousavi Nik:** None. **S. Hulsizer:** None. **I. Pessah:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

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Program#/Poster#: 407.10/K16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Title: The MED64-Quad II system increases throughput for studies of antiepileptic drug targets with *In vitro* MEA pharmacology on acute brain slices

Authors: S. YASUOKA[1], R. ARANT[2], *G. CHENG[2];
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Abstract: Micro-electrode arrays (MEAs) have been widely utilized to measure neuronal activities *in vitro*. The MEA technology offers many unique advantages to investigate neuronal circuitry, interaction and models of learning and memory, development, aging, disease and neurotoxicity. While several high-throughput platforms have been utilized for drug screening with cultured cell applications in recent years, there have been limited platforms designed for acute and culture slice applications. Here we present the capabilities of the MED64-Quad II system, a novel medium-throughput MEA designed specifically for acute or cultured slice applications. Recordings were made from acute hippocampal slices from 6-8 week old male ICR strain mice and the extracellular signals were obtained at 16 electrodes per slice (4 slices recorded simultaneously) or 64 electrodes per slice with MED64-basic system. Spontaneous spikes were recorded with 4 slices simultaneously using the MED64-Quad II System. On a given experiment of 16 or 64 electrodes recording, spontaneous firing were recorded from hippocampal and entorhinal cortex. The firing frequency per channel and the number of synchronized bursts were analyzed. Pharmacological agents NMDA, bicuculline, kainate, AP5,

CNQX, PTZ (pentylenetetrazole), and VPA (sodium valproate) were tested on the slices using MED64 system. The synchronized bursts were seen following bath perfusion of drugs. Antiepileptic drugs reduced the burst discharges. The results of this study indicated that the MED64-Quad II and Basic system increases throughput while maintaining high sensitivity to detect spontaneous spiking signals. It is a useful tool for drug discovery, target validation, compound screening for antiepileptic drug targets and pharmacological studies in acute brain slice applications *in vitro*.

Disclosures: **S. Yasuoka:** None. **R. Arant:** None. **G. Cheng:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

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Program#/Poster#: 407.11/K17

Topic: B.11. Epilepsy

Title: Neuroactive Steroids exhibit synergistic interactions with barbiturates at the $GABA_A$ receptor *In vitro* and this impacts on activity in an animal model of seizure

Authors: *M. A. ACKLEY, G. M. BELFORT, R. S. HAMMOND, M. C. QUIRK, G. MARTINEZ-BOTELLA, F. G. SALITURO, A. J. ROBICHAUD, J. J. DOHERTY;
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Abstract: Allopregnanolone and certain other second generation neuroactive steroids (NAS) are positive allosteric modulators of $GABA_A$ receptors, and are currently being studied for the treatment of seizure disorders such as super refractory status epilepticus and orphan genetic epilepsies. These compounds are believed to bind to one or more sites that are distinct from other classical modulators of $GABA_A$ receptors and as such are capable of modulating a broader range of subunit combinations than for example, benzodiazepines. We therefor sought to evaluate whether NAS could interact with other modulators at the level of the $GABA_A$ receptor and whether this interaction could amplify the activity of NAS in aborting seizures *in vivo*.
Whole cell patch clamp recordings were made from cells that heterologously expressed $GABA_A$ receptors. Allopregnanolone produced a concentration-dependent potentiation of GABA currents in cells expressing recombinant $\alpha_4\beta_3\delta$ subunits, which was 16,000-fold more potent than the modulation by pentobarbital. In the presence of a low concentration of pentobarbital the concentration-response curve of allopregnanolone was enhanced with an increase in the E_{max}, indicating a potential synergistic interaction. Construction of isobolographs confirmed that the interaction between allopregnanolone and pentobarbital was indeed synergistic.
To understand if this synergistic interaction would have a physiological impact, we assessed the

ability of NAS with or without phenobarbital to inhibit epileptiform discharges (ED) in brain slices. A synthetic NAS, SGE-516 inhibited ED in a concentration-dependent manner and in the presence of an inactive concentration of phenobarbital, the concentration-response curve was shifted to the left indicating an increased potency of SGE-516.
We next chose to assess whether NAS interacted with pentobarbital *in vivo*. Status epilepticus was induced in rats using lithium-pilocarpine and seizure activity was monitored using EEG. Allopregnanolone (0.3-15 mg/kg IV) reduced Spike Probability (SP), indicating a suppression of seizure activity. Plasma concentrations required to significantly reduce SP were in excess of 18 μM. When administered in the presence of a sub-active dose of pentobarbital (10mg/kg), the concentration of allopregnanolone required to significantly reduce SP, decreased 16-fold.
These data suggest that naturally occurring and synthetic NAS such as allopregnanolone and SGE-516 may interact with common modulators of $GABA_A$ receptors in a synergistic manner and support further study to determine if this interaction can result in an enhanced anti-convulsant potency *in vivo*.

Disclosures: **M.A. Ackley:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **G.M. Belfort:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **R.S. Hammond:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **M.C. Quirk:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **G. Martinez-Botella:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **F.G. Salituro:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time): SAGE Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time): SAGE Therapeutics.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 407.12/L1

Topic: B.11. Epilepsy

Support: NINDS Grant NS082644

Title: Partial activation of TrkB receptors corrects interneuronal calcium channel dysfunction and reduces epileptogenic activity in neocortical circuits following injury

Authors: ***F. GU**, I. PARADA, T. YANG, F. LONGO, D. PRINCE;
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Abstract: Decreased GABAergic inhibition due to dysfunctional inhibitory interneurons plays an important role in posttraumatic epileptogenesis. We previously showed that reduced N-current Ca^{++} channel (N-channel) function in GABAergic terminals contributes to interneuronal abnormalities and neural circuit hyperexcitability in the partial neocortical isolation ("undercut" or "UC") model of cortical injury. Because of the known role of BDNF in development and maintenance of interneurons, we tested the hypothesis that chronic TrkB activation would correct N channel abnormalities and suppress neural circuit hyperexcitability in the UC model of posttraumatic epileptogenesis. PTX BD4-3 (BD), a partial agonist at the BDNF TrkB receptor, was injected in UC rats (50mg/kg, once a day, i.p.) for 7 days beginning on the day of injury. Immunocytochemistry and confocal microscopy were used to assess the density of N and P/Q channels in presynaptic GABAergic terminals. Whole cell patch clamp recordings of evoked inhibitory postsynaptic currents (eIPSCs) from layer V pyramidal cells, together with effects of bath-applied N and P/Q channel blockers ω-conotoxin and agatoxin were used to determine effects of BD on N and P/Q channel function, respectively. To determine whether BD can decrease injury-induced circuit hyperexcitability, we treated control and UC rats *in vivo* with 45mg/kg pentylenetetrazole (PTZ) i.p 10 days after 7 days of saline or BD treatment and compared the incidence of EEG and behavioral seizures between different groups. Results showed that 1) chronic BD treatment up-regulated both N- and P/Q channel-immunoreactivity in GABAergic terminals; 2) the reduction in eIPSC amplitude after either N channel or P/Q channel blockade was increased in BD-treated UC rats, indicating enhanced function of both N and P/Q channels in the interneurons of UC cortex; and 3) BD treatment reduced the susceptibility to PTZ-induced seizures in UC rats. Results suggest that chronic TrkB activation with a partial agonist could be a promising strategy to rescue the injury-induced calcium channel abnormalities in inhibitory presynaptic terminals, thereby improving interneuronal function and suppressing circuit hyperexcitability following cortical trauma.
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Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: B.11. Epilepsy

Support: CURE Infantile Spasms Research Initiative

Title: A critical developmental window for 17β-estradiol antiepileptogenic effect in a mouse model of x-linked infantile spasms

Authors: *M. SIEHR, R. LUCERO, J. LALONDE, J. NOEBELS;
Neurol., Baylor Col. of Med., Houston, TX

Abstract: X-linked Infantile Spasms Syndrome (ISSX) is a catastrophic childhood epilepsy disorder. There are few treatments available that effectively reduce motor spasms in infants, prevent epilepsy later in life and improve developmental outcomes in children with ISSX. A common genetic cause of ISSX is a triplet-repeat expansion mutation in the *Aristaless-related homeobox* (*ARX*) gene. Our lab generated a mouse model with this mutation and $Arx^{(GCG)10+7}$ mice recapitulate many phenotypic features of ISSX, including neonatal motor spasms and epilepsy. ARX is a crucial transcription factor for the development of cortical GABAergic interneurons and $Arx^{(GCG)10+7}$ mutant mice have significantly reduced numbers of cortical GABAergic interneuron subtypes. Recently, our lab demonstrated that a one-week treatment of 17beta-Estradiol (E2) (40ng/g/day) administered to neonatal (P3-10) mutant mice reduced spasms in neonates and seizures in adults. Interestingly, E2 treatment in neonatal mutants also restored numbers of GABAergic interneuron subtypes in adults. This effect was age-dependent, as treatment of adults (P33-40) with E2 had no effect on these phenotypes. These results indicate that only early administration of E2 may have an antiepileptogenic effect in the $Arx^{(GCG)10+7}$ model. In order to effectively translate this therapy to the clinic, we must further define this critical developmental window. In this work, we aimed to define the temporal boundary for effective E2 treatment by delaying treatment initiation from P3 until P7. Delaying E2 treatment until the second postnatal week (P7-13) had no effect on seizures or interictal spikes (a second measure of cortical hyperexcitability) in adult $Arx^{(GCG)10+7}$ mice. These results indicate that there is a critical developmental window for antiepileptogenic effect of E2 in the *Arx* model of ISSX. Little is understood about the molecular mechanism of the antiepileptogenic effects of E2 and the basis for the critical therapeutic window. As estrogen receptor beta (ERb) is expressed in developing interneurons, we began by exploring whether activation of this single estrogen receptor subtype is sufficient to reproduce the effect seen with E2. Selective activation of ERb using LY500307 (Eli Lily & Co.) at 200ng/g/day from P3-10 was sufficient to produce a partial antiepileptogenic effect in $Arx^{(GCG)10+7}$. Treatment with LY500307 significantly reduced seizures but not neonatal spasms or interictal spikes. Full effect may require activation of other ERs. We aim to further elucidate the cellular and molecular mechanisms underlying the antiepileptogenic and neuroprotective effects of E2 in the $Arx^{(GCG)10+7}$ model of ISSX.

Disclosures: **M. Siehr:** None. **R. Lucero:** None. **J. Lalonde:** None. **J. Noebels:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Topic: B.11. Epilepsy

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VIEP-BUAP 2016

CA Neuroendocrinology BUAP-CA-288

YSV is fellowship from VIEP

Title: Valproic acid decreases the absence seizures in a myelin mutant taiep rat: A new animal model of epilepsy

Authors: *M. CORTES, Y. SILVA, J. R. EGUIBAR;
B. Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: Taiep rat is a new model of absence seizures that showed an initial hypomyelination followed by a progressive demyelination of central nervous system. The phenotype is inherited in an autosomal recessive manner. In electroencephalographic 24 hours recordings taiep rats have cortical spike-wave discharges (SWD) with a peak in the theta band frequency at 6.5 Hz, and occurs spontaneously along 24h. In the present study, we analyzed the effects of oral administration of 300 mg/kg of valproic acid on absence seizures and evaluate them during continuous electrographic recordings along circadian cycle. The rats were maintained in experimental room with a 12/12 light-dark cycle with the lights on at 0700. Rats were free access to rodent pellets and purified water. Rats were anesthetized by i.p. injection of ketamine-xylazine mixture and placed in a stereotaxic apparatus (Kopft). Three stainless steel screws were implanted under aseptic conditions for electroencephalographic recordings, and a bipolar electrode in the CA1 field of the hippocampus and also Ni-chromium electrodes for electrooculogram and electromyography recordings. All signals were digitalized, filtered and stored in a hard disk of PC computer and analyzed using Harmonie software (Stellate). After valproic acid by oral administration in male taiep rats showed less frequency of SWD ($P<0.05$), and also a diminution in their mean duration ($P<0.05$). These effects lasted more than 8h suggesting that they are more sensible to this prototypical antiepileptic drug in a similar way as WAG/Rij and GAERS rats. In conclusion, taiep rats are a suitable model of absence seizure and also to test new antiabsence drugs.

Disclosures: **M. Cortes:** None. **Y. Silva:** None. **J.R. Eguibar:** None.

Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

Location: Halls B-H

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CURE (Infantile Spasms Initiative)

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the Abbe Goldstein/Joshua Lurie and Laurie Marsh/ Dan Levitz families

Title: Acute effects of lacosamide on spasms in the multiple-hit rat model of infantile spasms

Authors: *O. SHANDRA[1], W. B. MOWREY[2], S. L. MOSHÉ[1,3,4], A. S. GALANOPOULOU[1,3]; [1]Saul R. Korey Dept. of Neurology, Lab. of Developmental Epilepsy, [2]Dept. of Epidemiology and Population Hlth., [3]Dominick P. Purpura Dept. of Neuroscience, Montefiore/Einstein Epilepsy Ctr., [4]Dept. of Pediatrics, Albert Einstein Col. of Med., Bronx, NY

Abstract: BACKGROUND: Infantile Spasms (IS) are age-specific epileptic seizures of West syndrome, associated with severe cognitive and neurodevelopmental deficits, high risk of early mortality and intractable epilepsy development. Outcomes might be partially improved by early cessation of spasms with effective therapy. We used a multiple-hit rat model to recreate medically refractory IS due to structural lesions and to identify new therapeutic agents for IS with rapid effect on spasms.
OBJECTIVE: Determine whether a single injection of an antiepileptic drug lacosamide, used for treatment of other types of seizures, can produce rapid cessation of spasms in the multiple-hit rat model of IS.
METHODS: To induce the spasms, male Sprague-Dawley rats received right intracerebral injections of doxorubicin and lipopolysaccharide on postnatal day (PN) 3 and intraperitoneal (i.p.) injection of p-chlorophenylalanine on PN5. Daily milestones of the rats were scored using a battery of neurodevelopmental tests. Intermittent video monitoring of the rats was done on PN4 (1 hour pre-drug injection and 5 hours post-drug injection) and on PN5 (2 two-hour sessions). Rats were sacrificed for histology on PN5 after last monitoring session. A single i.p. injection of lacosamide (10, 30 and 50 mg/kg) or vehicle was given after the onset of spasms on PN4 in a randomized, blinded, vehicle-controlled, dose-response study. A linear mixed model analysis of raw or normalized log-transformed spasm rates was used, considering the repeated observations on each individual animal. 12-15 rats per group were studied.

RESULTS: All tested doses of lacosamide, given as single injection after the onset of spasms, produced a rapid cessation of spasms within the first post-injection hour. Significant reduction of spasms was seen at the 4th hour (seen with the 30 and 50 mg/kg/i.p. dose) and 5th hour (seen with the 30mg/kg dose; borderline effect seen with the 50mg/kg i.p. dose). All doses were well tolerated. Neurodevelopmental outcomes were unaffected.
DISCUSSION: We provide proof-of-concept evidence that a single injection of lacosamide given after the onset of spasms can acutely suppress the spasms in the multiple-hit rat model of IS. The ongoing studies include the correlation of the efficacy of lacosamide with its pharmacokinetics and video-EEG recordings analysis. Our future goal is to test the effects of lacosamide on spasms, long-term neurodevelopmental, cognitive and epilepsy outcomes after repeat administration in the multiple-hit rat model of IS.

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Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Topic: B.11. Epilepsy

Support: 1R21TW009384-01

NC123240.1

Title: Transcranial focal electrical stimulation via concentric ring electrodes in freely moving cats, antiepileptogenic and postictal effects

Authors: *A. VALDÉS-CRUZ[1], B. VILLASANA-SALAZAR[2], W. G. BESIO[3], V. M. MAGDALENO-MADRIGAL[2], D. MARTÍNEZ-VARGAS[2], S. ALMAZÁN-ALVARADO[2], R. FERNÁNDEZ-MAS[2];
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Abstract: Temporal lobe epilepsy exhibits a high resistance to pharmacological treatments. Transcranial focal electrical stimulation (TFS) via tripolar concentric ring electrodes (TCRE) is a non-invasive experimental proposal to treat pharmacoresistant epilepsy. Thus the aim of our study was to investigate the effect of preventive- and responsive-TFS application via TCREs on

convulsive activity induced by electrical amygdaloid kindling (AK) model of TLE epileptogenesis, which consists in repeated and periodical electrical stimulations to limbic brain structures, leading progressively to the induction of an electroencephalographic afterdischarge. Therefore we investigated the effect of TFS on seizure activity by stimulating over the kindled area in cats. In addition, spectral power analysis of electrographic activity induced by TFS was also explored. Fifteen adult cats were implanted in both amygdalae (AM) and prefrontal cortices (PFC) and assigned to three experimental groups: Control, only received AK; preventive-TFS group in which a TCRE was placed over the vertex, TFS (300 Hz, 200 μs biphasic square pulses) was delivered for 40 minutes prior to AK. Responsive-TFS group, with the TCRE over the temporal bone ipsilateral to the kindled AM, TFS was administered for 2 minutes after the AK onset for 40 days, thereafter only AK was applied. Daily AK was applied (60 Hz, 1 ms monophasic square pulses for 1 s, 300-500 μA) until all animals reached kindling stage VI. Spectral power analysis was performed in frequency bands 1-4 Hz, 4-9 Hz, 10-14 Hz and 15-30 Hz on signals from both PFC and AM. Responsive-TFS inhibited the kindling development. This suppressive effect was long-lasting with the Responsive-TFS group remaining at the focal seizure stages for the next 20 days after the TFS cessation, and needed 80.0 ± 15.42 AK stimulations to reach stage VI. Preventive-TFS did not protect against epileptogenesis. Nonetheless, it produced a continued decrease in postictal spectral power. In conclusion TFS applied during seizures over the epileptogenic area may exhert a therapeutic effect against intractable epilepsy with the advantage of its non-invasive application.

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Poster

407. Epilepsy: Anticonvulsant and Antiepileptic Strategies

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Topic: B.11. Epilepsy

Support: NC 3140.1

Title: Effects of High- and Low-frequency stimulation of thalamic reticular nucleus on ptz-induced seizures in rats

Authors: *V. M. MAGDALENO-MADRIGAL, A. VALDÉS-CRUZ, D. MARTÍNEZ-VARGAZ, S. ALMAZÁN-ALVARADO, R. FERNÁNDEZ-MAS;
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Abstract: The use of electrical stimulation therapy for epilepsy is currently being studied in experimental animals and patients. We have reported that the high-frequency stimulation (HFS) in the thalamic reticular nucleus (TRN) induced an anti-epileptogenic effect. Our study was designed to evaluate the effects of HFS and low-frequency stimulation (LFS) applied in the TRN on the expression of pentylenetetrazole-induced (PTZ) seizures. Experiments were performed using Wistar male rats, with electrodes stereotaxically implanted in the left TRN (AP -1.4mm, L 1.6mm, H 6.2mm). Epidural EEG recording screws were implanted in the prefrontal cortex for EEG recording. The rats were classified as follows: Control group, which received only PTZ injection; HFS+PTZ group, which received HFS prior to PTZ; LFS+PTZ, which received LFS prior to PTZ; PTZ+HFS, which received HFS after the PTZ injection; and PTZ+LFS, which received LFS after the PTZ injection. EEG recordings were obtained from the cortex and were evaluated to assess ictal behavior over 30 min. HFS and LFS in the TRN induced a significant decrease in seizure severity. Additionally, pre-treatment with HFS and LFS protected against death as a consequence of *status epilepticus*. The spike-wave complex frequency was modified in both HFS and LFS protocols. These data further highlight the role of TRN in mediating the expression of seizures and provides experimental support for the concept that this thalamic region may be a promising target for focal stimulation to treat intractable seizures in humans.

Disclosures: V.M. Magdaleno-Madrigal: None. **A. Valdés-Cruz:** None. **D. Martínez-Vargaz:** None. **S. Almazán-Alvarado:** None. **R. Fernández-Mas:** None.

Poster

408. Epilepsy: Human Studies I

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 408.01/L7

Topic: B.11. Epilepsy

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Doris duke charitable foundation

Title: Characteristics and consistency of seizure offset dynamics in humans

Authors: *D. N. CRISP[1], J. SCOTT[1], M. COOK[3], M. DUEMPELMANN[4], G. WORRELL[5], W. STACEY[2];
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Abstract: Our recent model of seizure dynamics showed that seizure offsets can be described by a limited set of four possible behaviors that are well characterized in bifurcation theory [1]. We investigated the prevalence of each of these bifurcations in a set of 85 patients from 4 different centers, and found that within the population the most common offset dynamics are logarithmic (75%) and constant (15%) scaling of interspike intervals (ISI). In this work, we examine the consistency of seizure dynamics within patients recorded over several months and explore the dynamics of very long seizures. We measured the ISI of 985 seizures in 13 patients. Each of the 13 patients has a distribution of both logarithmic and constant scaled seizures, all with nearly the same 80% prevalence of logarithmic dynamics. Several patients had distinct populations of seizures with different lengths [2], each displaying different parameter distributions. In contrast, we analyzed 50 long seizures (> 2 minutes), and found that they were predominantly characterized by constant ISI (80%). One episode of subclinical status epilepticus lasting almost 60 minutes was characterized by multiples episodes of waxing and waning ISI. This seizure displayed multiple bifurcations that transitioned into irregular spiking before restarting a different bifurcation. It also contained multiple brief episodes of constant ISI with zero variance, which were never seen in shorter seizures. Together, these findings indicate individual patients have similar prevalence of each bifurcation type as in the general population for normal seizures. In contrast, longer seizures tend to have different dynamics. These findings indicate that bifurcation theory is a valuable tool to describe human epilepsy.

Disclosures: D.N. Crisp: None. **J. Scott:** None. **M. Cook:** None. **M. Duempelmann:** None. **G. Worrell:** None. **W. Stacey:** None.

Poster

408. Epilepsy: Human Studies I

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Program#/Poster#: 408.02/L8

Topic: B.11. Epilepsy

Title: Comparing interictal discharges from intracranial EEG in patients with and without epilepsy

Authors: *B. N. LUNDSTROM, G. WORRELL, M. STEAD;
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Abstract: Introduction: Approximately 1% of people have epilepsy and seizures reoccur in an unpredictable fashion leading to significant morbidity. While interictal epileptiform discharges (IEDs) recorded with EEG, such as spikes and sharp waves, provide localizing information about the seizure focus, they generally are not found to correlate with an increased seizure probability.

We recently described epilepsy patients for whom the rate of IEDs recorded by intracranial EEG (iEEG) appears to be correlated with long-term seizure probability. Thus, the rate of IEDs may have more clinical relevance when recorded by iEEG rather than scalp EEG. This raises the question to what extent typically defined IEDs are specifically associated with epilepsy. Here, we examine the rates of interictal transients detected using a validated automated detector for IEDs in patients who underwent iEEG monitoring due to intractable facial pain. **Methods:** Patients without a history of seizures underwent iEEG monitoring with subdural electrodes prior to placement of cortical stimulating electrodes for treatment of facial pain. Transient EEG events were detected as IEDs automatically in six 15-minute blocks using a previously validated method (Barkmeier et al.) in five electrodes per patient. These data were compared with iEEG data gathered from epilepsy patients in high and low seizure probability states (Lundstrom et al.). **Results:** Preliminary results from a total of 15 hours of data from two patients show an average discharge rate of 0.06 per second (SD 0.03). The mean rate in electrodes distant to the seizure focus in epileptic patients was 0.14 per second (SD 0.11). The mean rate in electrodes near the seizure focus was 0.61 per second (SD 0.07) prior to treatment and 0.08 per second (SD 0.07) following treatment. **Discussion:** Although the rate of IEDs is very low in scalp EEG recordings of patients without epilepsy, our results suggest that it may not be negligible in iEEG recordings. The rates of iEEG transients classified as IEDs using an automated detector in patients without epilepsy is comparable to IED rates of treated epileptic patients. This raises the possibility that at least some transient EEG fluctuations similar to IEDs in patients with epilepsy may occur in patients without epilepsy, and these waveforms are not specific to epileptic mechanisms at the cellular level. One explanation is that these findings relate to cortical injury and the impact of implanted subdural grids on cortex. **References:** Barkmeier, D. T. *et al. Clin. Neurophysiol.* 123**,** 1088-95 (2012). Lundstrom, B. N. *et al.* Chronic subthreshold cortical stimulation to treat focal epilepsy, submitted.

Disclosures: B.N. Lundstrom: None. **G. Worrell:** None. **M. Stead:** None.

Poster

408. Epilepsy: Human Studies I

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 408.03/L9

Topic: B.11. Epilepsy

Title: Mechanisms of widespread cortical fMRI increases and decreases in absence seizures

Authors: *Y. CHEN[1], S. BRAUN[1], J. GUO[1], H. BLUMENFELD[1,2,3];
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Abstract: It is hoped that an extensive understanding of the neuronal activity changes during spike-and-wave discharge (SWD) might lead to improved treatment for absence seizures. Blood oxygen-level dependent (BOLD) fMRI signal change is not directly related to neuronal activity as measured by EEG. The physiological basis for early fMRI increases and sustained decreases remains unknown. A standard hemodynamic response function (HRF) is commonly used to relate electrical brain activity to the expected fMRI timecourse. An important question is whether the small fMRI increases before seizures and sustained decreases after absence seizures can be explained by any electrical activity pattern when using the standard HRF. We created a signal processing model of electrical activity by deconvolving the BOLD signal with the canonical HRF. Activated neural activity before seizures and deactivated neural activity after seizures suggest that if the canonical HRF is correct, then the small increases before seizures and decrease in BOLD fMRI signals following seizures can't be explained by electrical activity during the seizure alone. Also we customized the HRF by using ictal period boxcar neural activity model alone. We also evaluated that neural activity changes occur only during the period of SWD. The electrical activity is modeled with a simple boxcar representing EEG seizure. To fit the data, this approach yielded customized HRFs which were markedly different from the canonical HRF including small increases of the response function before and profound decreases after EEG seizure times. With further investigation it is hoped that the full pathophysiology of neuronal changes before, during and after SWD can be better understood to help guide future treatment.

Disclosures: Y. Chen: None. **S. Braun:** None. **J. Guo:** None. **H. Blumenfeld:** None.

Poster

408. Epilepsy: Human Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 408.04/L10

Topic: B.11. Epilepsy

Title: Driving simulation testing of patients with epilepsy during inpatient video/EEG monitoring

Authors: L. GOBER[1], Y. SI[1], G. TOULOUMES[1], W. CHEN[1], E. MORSE[1], R. GEBRE[1], A. BAUERSCHMIDT[1], M. YOUNGBLOOD[1], C. CUNNINGHAM[1], C. EZEANI[1], Z. KRATOCHVIL[1], J. BRONEN[1], J. THOMSON[1], K. RIORDAN[1], L. HIRSCH[1], *H. BLUMENFELD[2,3,4];

[1]Neurol., [2]Neurology, Neurobiology, & Neurosurg., [3]Neurosci., [4]Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Epileptic seizures can significantly limit the ability of affected individuals to lead normal lives, especially when loss of consciousness occurs. Often, epileptic individuals are restricted from driving until it can be shown that seizures are fully controlled. In the U.S. driving license issuance is dependent on people with epilepsy maintaining a seizure-free period. Consequently, physicians play an important role in determining whether or not the patient should be allowed to drive. However, decisions about driving can be difficult due to the lack of objective data available about patient driving performance during the ictal and post-ictal periods of clinical seizures. In addition the potentially important effects of subclinical epileptiform discharges on driving safety are not known. In this study, we analyze ictal and interictal driving data captured prospectively from a driving simulator in patients undergoing video/EEG monitoring in the epilepsy monitoring unit at Yale New Haven Hospital. Performance data is analyzed with attention to ictal and interictal periods of play. A total of 33 seizures in 20 patients were analyzed, along with 143 subclinical epileptiform discharges in 9 patients. Baseline was defined as interictal driving performance and was compared to ictal driving performance through quantitative analysis of car velocity, steering wheel movement, application of the brake pedal and crash occurrence during the ictal and postictal periods as well as during subclinical epileptiform discharges. We found variable impairment both during seizure/postictal periods as well as during subclinical epileptiform discharges with some showing obvious impairment and others no change in driving performance. In ongoing work we hope to expand upon our current analysis of performance variables as well as determine whether specific seizure types or localizations present a greater driving risk, with the goal of providing improved guidance to physicians and patients with epilepsy.

Disclosures: L. Gober: None. **Y. Si:** None. **G. Touloumes:** None. **W. Chen:** None. **E. Morse:** None. **R. Gebre:** None. **A. Bauerschmidt:** None. **M. Youngblood:** None. **C. Cunningham:** None. **C. Ezeani:** None. **Z. Kratochvil:** None. **J. Bronen:** None. **J. Thomson:** None. **K. Riordan:** None. **L. Hirsch:** None. **H. Blumenfeld:** None.

Poster

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Support: James G. Hirsch M.D., Endowed Medical Student Research Fellowship

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Title: Frontal lobe seizures and impaired consciousness: intracranial eeg markers.

Authors: *R. GEBRE[1], M. DHAKAR[1], E. GROVER[1], I. QURAISHI[1], E. STERNBERG[1], I. GEORGE[1], A. SIVARAJU[1], J. BONITO[1], H. ZAVERI[1], L. GOBER[1], S. AHAMMAD[1], S. GHOSHAL[1], L. HIRSCH[1], D. D. SPENCER[2], J. L. GERRARD[2], H. BLUMENFELD[1,2,3]; [1]Dept. of Neurol., [2]Dept. of Neurosurg., [3]Dept. of Neurosci., Yale Sch. of Med., New Haven, CT

Abstract: Loss of consciousness is an important morbidity associated with epileptic seizures, and understanding how altered consciousness occurs could have impact on future therapies. Consciousness includes multiple levels of input and output that maintain alertness, attentiveness, and awareness of both self and the environment. Previous work from our laboratory using SPECT imaging and intracranial EEG analysis of temporal lobe seizures has supported the network inhibition hypothesis. This states that impaired consciousness in temporal lobe epilepsy involves activation of the temporal lobe leading to abnormal activity in the thalamus and brainstem subcortical arousal systems. These changes lead to the depressed function in the frontal and parietal association cortices and impaired consciousness. Research on the mechanisms of loss of consciousness in frontal lobe epilepsy is not yet as well defined. We initially examined the intracranial EEG in 9 focal frontal seizures from 6 patients that had impaired consciousness and noted that there was a common ictal pattern of widespread low voltage fast activity. We hypothesized that widespread low voltage fast activity may play a role in loss of consciousness in patients with frontal lobe seizures. To further investigate this hypothesis we obtained a larger cohort of 91 patients from the Yale epilepsy surgery program. We then included patients that had intracranial studies that found a frontal lobe onset, and excluded seizures with no meaningful behavioral interaction and also eliminated seizures that secondarily generalized. This led to a final cohort of 16 patients who were recorded from 2004-2015 and 50 seizures. In ongoing analyses, behavioral responsiveness during the ictal period will be scored while being blinded to the EEG recordings. Likewise, intracranial EEG recordings will be scored while being blinded to the videos of the seizure behaviors. These investigations should enable us to determine whether widespread low voltage fast activity or a different mechanism may cause widespread altered brain function and impaired consciousness in frontal lobe seizures.

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Poster

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Hungarian Brain Research Program grant KTIA-13-NAP-A-IV/1-4,6.

Title: Synergism of cross-frequency coupling and phase synchrony in epileptic focus localization: an iEEG study

Authors: *T. NÁNÁSI[1,2], B. FILE[3], L. ERőSS[1], L. ENTZ[1], D. FABÓ[1], I. ULBERT[2,3];
[1]Natl. Inst. of Clin. Neurosciences, Budapest, Hungary; [2]Insitute of Cognitive Neurosci. and Psychology, RCNS, HAS, Budapest, Hungary; [3]Fac. of Information Technol. and Bionics, Pázmány Péter Catholic Univ., Budapest, Hungary

Abstract: Background: Pharmacologically intractable epilepsy is a neurological disorder that has enormous impact on the quality of life of the patients. The therapeutic workflow involves intracranial electroencephalography (iEEG) recordings and surgical removal of the resection site (RS), which ideally overlaps with the epileptogenic zone (EZ). Determining the EZ is therefore a critical step. Clinical EZ detection relies extensively on peri-ictal recordings, which have to be collected in sufficient number and quality, usually resulting in more than one week of hospitalization with drug withdrawal and therefore poses heavy burden for the patients.
Methods: We used previously acquired clinical and subdural grid iEEG data of five patients suffering from drug resistant focal epilepsy. Electrode positions were reconstructed from preoperative MRI, and post-operative CT scans. Four 3 minute long segments of recordings free from any obvious epileptic activity with at least 1 hour of temporal separation from actual seizures were analyzed for each patient. The patients were sleeping during the acquisition. After band-pass filtering, Phase Amplitude Coupling (PAC) and Phase Lag Index (PLI) values were calculated and phase-synchronized phase-amplitude coupling constellations (PoP) were considered. Also, a heuristic approximation of spectral entropy (DASE) was assessed. The dimensionalities of the measurements were reduced using the node strength parameter of the resulting graphs, and then those per-electrode values were evaluated for EZ localization potential.
Results: Node strength maps delivered from the novel measurements (PoP and its combination with DASE) showed maximum values and higher averages inside the RS in the case of the four successful surgeries, whereas in the case of the patient with residual seizures the delivered maximum values and higher averages pointed outside the clinically applied RS.
Conclusions: In this pilot study we demonstrated the potential usefulness of phase-synchronized

phase-amplitude coupling constellations as a hybrid construct from well-established PAC and PLI coupling measurements in automatized EZ localization. Also, we introduce a heuristic approximation of spectral entropy which can further improve the specificity of EZ detection. Our analysis works on seizure-free sleep iEEG data abundant in a typical clinical setting and aside from the initial frequency band definitions operates in a threshold-free manner.

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Poster

408. Epilepsy: Human Studies I

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Dr. Engel by NS033310

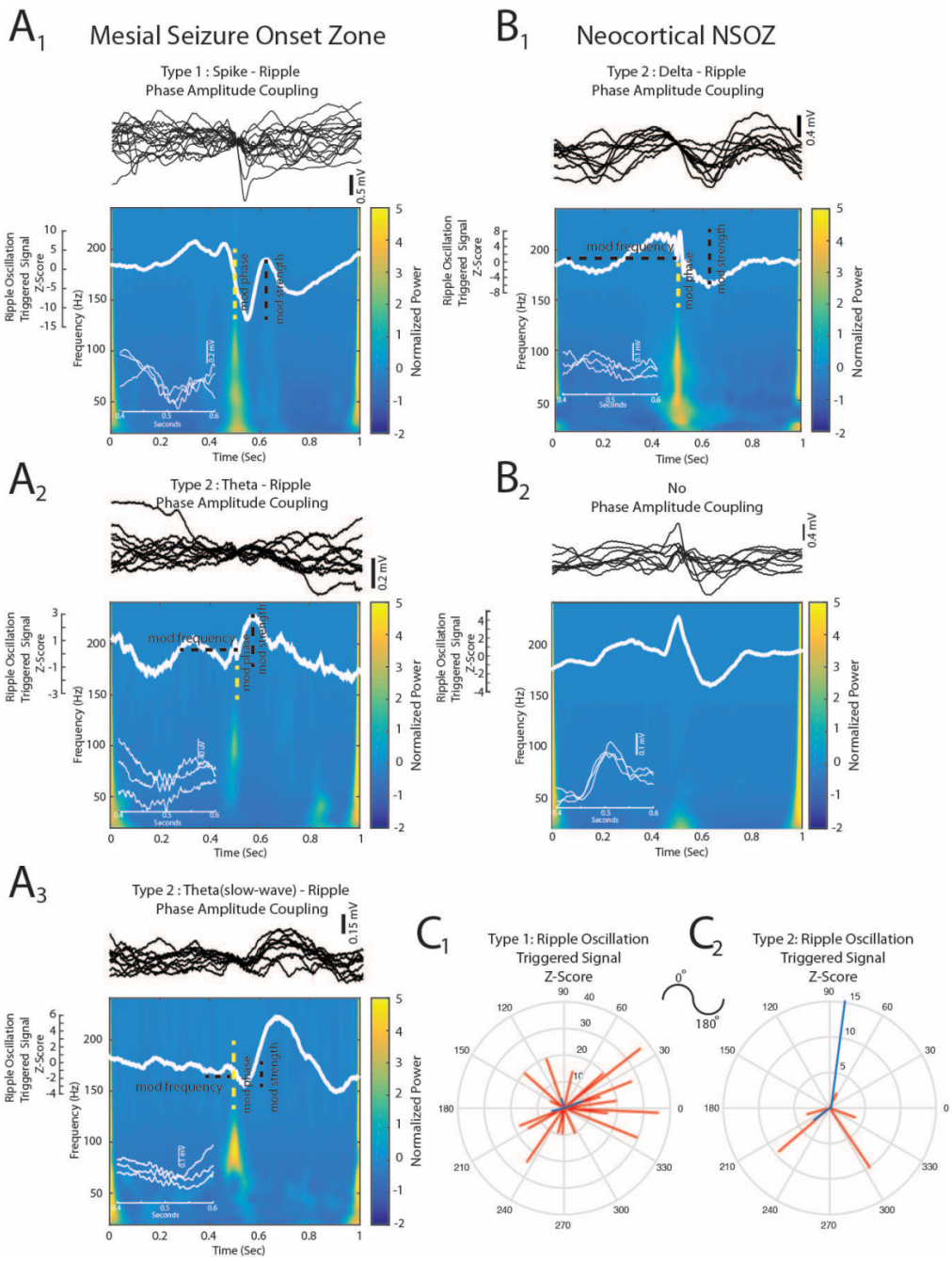
Dr. Staba by NS071048

Dr. Bragin by NS065877

Title: Ripples show increased phase-amplitude coupling in mesial temporal lobe epilepsy seizure onset zones

Authors: *S. A. WEISS[1], I. OROSZ[2], S. MOY[3], L. WEI[3], M. VAN'T KLOOSTER[4], R. T. KNIGHT[5], R. F. HELFRICH[5], R. M. HARPER[3], A. BRAGIN[3], I. FRIED[3], J. ENGEL, Jr.[3], R. STABA[3];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Neuroradiology, [3]Univ. of California Los Angeles, Los Angeles, CA; [4]Dept. of Neurol. and Neurosurg., Brain Ctr. Rudolf Magnus, Utecht, Netherlands; [5]Univ. of California Berkeley, Berkeley, CA

Abstract: High frequency oscillations (HFO, 80-150 Hz; “ripples”) accompany learning and memory processes, but are also an epileptogenic biomarker. Differences between normal and pathological ripples are unclear, but we hypothesized that pathological events exhibit increased coupling between low-frequency EEG phase and ripple amplitude. We examined ripple phase-amplitude coupling (PAC) during inter-ictal intracranial depth macroelectrode recordings from 11 patients with mesial temporal lobe epilepsy using a Hilbert detector; PAC was determined by 1) computer-aided visual inspection of iEEG and ripple band, 2) identifying the modulating signal using oscillation-triggered coupling (OTC) measures, and 3) measuring PAC using a phasor transform and correlating the resulting coupling strength and phasor rate with the seizure onset zone (SOZ). Visual inspection of the iEEG and the modulating signal using OTC methods revealed two forms of PAC; type-I, coupling between inter-ictal discharge phase and ripple amplitude, and type-II, coupling between delta or theta oscillation phase and ripple amplitude. PAC-I ripples were larger amplitude and lower in frequency compared to PAC-II ripples. Type-I and type-II PAC coupling strength and rate were both increased in the SOZ. Ripple and phase-locked ripple phasor rates exhibited an 82%, and 87.7% precision for the SOZ, respectively. The SOZ rate ratio, comparing mean event rates in SOZ and non-SOZ, for phase locked ripple phasors increased across all 11 patients, relative to the ripple SOZ rate ratio. Phase-locking strength also correlated with likelihood of electrode placement in the SOZ. These results suggest that pathological ripples are generated by two distinct mechanisms distinguished by increased PAC magnitude.



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Poster

408. Epilepsy: Human Studies I

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Topic: B.11. Epilepsy

Support: Arizona Biomedical Research Commission

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Title: An algorithmic adjunct to visual inspection: using an Empirical Mode Decomposition based algorithm to detect seizures

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Abstract: Objective: Visual inspection of electrocorticography (ECoG) data has remained the seizure identification and localization method of choice for over 65 years. Visual inspection, however, is subjective and lacks specificity and sensitivity. Computational algorithms can quickly and objectively find signal characteristics that may not be detectable with visual inspection. Many seizure detection algorithms assume stationarity or linearity, making them inappropriate for the analysis of biological data. Empirical mode decomposition (EMD) does not require linearity or stationarity and is data driven. We propose the use of EMD as a seizure detection tool to supplement the visual review process.
Methods: EMD is an adaptive sifting algorithm that iteratively breaks complex waveform data into component waveforms, called intrinsic mode functions (IMF). The number of IMFs produced during the decomposition process is indicative of the complexity of the original waveform, with respect to frequency and amplitude. An EMD based algorithm was developed and tested on data from eight patients undergoing ECoG monitoring for refractory epilepsy. The recorded data were broken down into 136 ten-minute long clips. There were 68 seizure clips that were centered on a clinically determined seizure onset times, and 68 associated non-seizure clips. Prior to applying EMD, the signals for each grid were common average re-referenced (CAR). The algorithm was windowed through the data clips in 5 s windows with a 3 s step size. Two thresholds were established, one for each individual electrode and one for all electrodes used in each patient. Electrographic events were identified by considering changes in the number of IMFs generated for each individual electrode. Potential seizures were marked based on patterns

in increases in the number of IMFs across the population of all electrodes in a given clip.
Results: Analysis is ongoing. Current methods produced an algorithm sensitivity ranging from 100.00% to 40.00% across patients, when compared to seizures marked by visual inspection. The mean false positive rate is 1.24 detections/hr. There are a total of 14 false negative detections, 12 of which occurred when there was a deep focus.
Conclusions: The results suggest that a biological data driven algorithm could serve as a useful tool to objectively identify changes in cortical activity associated with seizures. However, detection algorithms may need to be adapted to different electrode types or particular neural structures. A process based on an appropriate algorithm could objectify and standardize the reviews across centers, leading to improved patient care.

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Poster

408. Epilepsy: Human Studies I

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Topic: B.11. Epilepsy

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Title: Disruption of resting state networks by section of the corpus callosum in humans

Authors: *J. L. ROLAND[1], A. Z. SNYDER[2], C. D. HACKER[3], E. C. LEUTHARDT[1], M. D. SMYTH[1];
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Abstract: Functionally related areas of cerebral cortex maintain correlated time signals in blood oxygen level dependent (BOLD) magnetic resonance imaging (MRI). These well-organized areas constitute networks that are identified in resting state functional connectivity MRI (rsfcMRI) analysis. To advance our understanding of these cerebral networks we studied individuals undergoing section of the corpus callosum for medically refractory epilepsy. Our cohort consists of twenty-six pediatric patients who underwent rsfcMRI before and after undergoing transection of either the anterior two-thirds (N = 7) or complete (N = 19) corpus callosum for medically refractory epilepsy. Our results show canonical networks, with bilateral coherence before intervention, are disrupted immediately after callosotomy. Ipsilateral signal coherence is maintained bilaterally. This is a highly unique cohort of surgically lesioned callosotomy patients with rsfcMRI analysis pre- and immediately post-intervention. These data

implicate the corpus callosum as a means for network coherence via cortico-cortical communication opposed to thalamo-cortical modulation of resting state networks.

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Poster

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Epilepsy Clinic of the General Hospital of Mexico

Central Hospital of San Luis Potosí

State Mental Health Center of Queretaro

Magnetic Resonance Unit UNAM

Title: Association between white matter changes and cognitive deficits in patients with temporal lobe epilepsy

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Abstract: Temporal lobe epilepsy (TLE) is associated with white matter (WM) spread damage that extends beyond the epileptic focus (Otte el al; Epilepsia 2012). Furthermore, TLE patients also present cognitive impairments, which are related to structural changes through the brain (Dabbs et al; Epilepsy & Behavior 2009). Given that most cognitive abilities require an activation of several brain regions altogether, it is likely that the network abnormalities seen in TLE are responsible for cognitive problems.Our goal was to evaluate white matter structures in TLE patients, and to search for correlations between diffusion parameters of these structures and neuropsychological performance.
We included 18 Left TLE patients (L-TLE), 12 Right TLE (R-TLE) and 24 controls paired by

age, gender and years of studies. Images were acquired using a 3T scanner. Diffusion weighted images (DWI) were acquired with a resolution of 2x2x2 mm^3, 60 different diffusion gradient directions and one non-DWI volume.DWI where pre-processed and a diffusion tensor (DTI) model was fitted in order to obtain fraction anisotropy (FA) maps. The JHU-WM template was registered to the FA map to obtain 43 regions of interest (ROI) of the main WM fascicles. All subjects underwent full WAIS-IV and WMS-IV assessment. Each WM ROI was correlated with neuropsychological measures with an ANCOVA model per group. Statistical significance was defined as $p < 0.05$, corrected for multiple comparisons.
There were no significant differences of demographic variables between groups; neither between epilepsy groups in terms of age of seizure onset or number of anti-epileptic drugs. With respect to controls, R-TLE had significant differences in all cognitive areas evaluated ($p < 0.05$) while L-TLE only showed differences in 4 cognitive domains. Five regions of white matter showed a significant interaction with the group factor, and all of them showed a clear positive correlation only in the L-TLE group.
We found more extension of WM abnormalities and cognitive impairment in diverse neuropsychological scales in R-TLE group, as compared L-TLE.In both TLE groups the FA reduction was observed in associative fascicles such as uncinate and corpus callosum.
Conclusion: we found strong evidence of structural WM abnormalities that correlate with neuropsychological assessments in TLE patients. The spread WM alteration leads to a disrupted communication between different cerebral areas and therefore it impacts in the cognitive performance. The relation of structure with a specific cognitive performance helps to understand how chronic epilepsy spreads through the brain and it aids to get an accurate personal diagnosis and treatment.

Disclosures: **R. Rodríguez Cruces:** None. **V. Camacho:** None. **L. Concha:** None.

Poster

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Topic: B.11. Epilepsy

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Title: A prospective study of the prevalence of cell-surface neuronal autoantibodies in adult patients with recent-onset epilepsies of unknown aetiology

Authors: ***T. C. MOLONEY**[1,2], S. R. IRANI[2], J. ADCOCK[2], A. SEN[2], P. WATERS[2], A. VINCENT[2], B. LANG[2];

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Abstract: Background: The discovery of pathogenic autoantibodies, targeting neuronal surface proteins, has led to the concept of antibody-mediated diseases of the CNS. Various studies have found autoantibodies in approximately 10 % of patients with epilepsy, particularly in focal epilepsies of unknown cause. However, a formal study limiting patients to those with a recent diagnosis of focal epilepsy and search for novel cell-surface antigens needs to be performed. **Methods:** Two hundred and twenty four adults (113 female/111 male; age range 18-91) with newly-diagnosed focal epilepsies (<12 months of onset) were prospectively recruited from the Thames Valley region, United Kingdom. Participants' sera were tested for antibodies to glutamic acid decarboxylase (GAD), the voltage-gated potassium channel (VGKC)-complex and associated proteins, LGI1, CASPR2 and contactin-2, dipeptidyl-peptidase-like protein 6 (DPPX) and to the NMDA, AMPA, $GABA_B$, $GABA_A$ and glycine receptors. In addition, to observe novel surface antibodies, sera were tested for IgG binding to the surface of cultured neurons. **Findings:** Positive antibody titres were detected in 35 of 224 patients. Twenty-one of the positive samples had antibodies to the voltage-gated potassium channel-complex (VGKC-c); four patients were identified as being directed against known VGKC-c associated proteins LGI1 (n=2) and CASPR2 (n=2). A further two patients had additional autoantibodies to GAD or GABAb-receptors in conjunction with VGKC-c antibodies. Other antibodies detected included those to CASPR2 (n=2) and Tag1 (n=1) without associated VGKC-c antibodies, NMDAR (n=3), AMPAR (n=1), Glycine R (n=1) GABAaR (n=1) and GABAbR (n=1). Eleven sera showed positive IgG staining on rat hippocampal neurones and in 4 of these samples the antigen was not known. **Interpretation:** Detection of antibodies in 15.6 % of adult patients with new-diagnosed epilepsy, including 8.4 % with antibodies to neuronal surface antigens, adds substantially to previous studies that pointed to an antibody-mediated pathogenesis in a proportion of new-onset focal epilepsies in adults. Further research will be directed at improved understanding of clinico-serological correlation to enable identification of relevant clinical features that may better predict autoantibody-mediated epilepsy.

Disclosures: T.C. Moloney: None. **S.R. Irani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalty for antibody assays. **J. Adcock:** None. **A. Sen:** None. **P. Waters:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Received speaker honoraria from Biogen Idec and Euroimmun AG, Royalties for antibody assays. **A. Vincent:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AV receives royalties and payments for antibody assays and AV is the named inventor on patent application WO/2010/046716 WO/2010/046716 entitled 'Neurological Autoimmune Disorders'. **B. Lang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalties for antibody assays.

Poster

408. Epilepsy: Human Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 408.12/M4

Topic: B.11. Epilepsy

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Title: Ictal-like HFOs during interictal periods can identify the seizure onset zone

Authors: *W. C. STACEY[1], K. R. MOON[2], A. O. HERO, III[2], S. V. GLISKE[2];
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Abstract: High frequency oscillations (HFOs) are a biomarker of epileptic tissue, being developed to help guide resective surgery. One major challenge limiting clinical translation is that HFOs can occur due to both normal and epileptic processes. One potential method to distinguish between normal and epileptic HFOs is to compare “ictal-like” versus “interictal-like” HFOs. We hypothesize that healthy tissue can produce ictal-like HFOs, but that this should only occur when seizures have spread to that tissue. In contrast, we hypothesize that epileptic tissue may have “ictal-like” HFOs at any time. In order to classify HFOs, we used a validated automated algorithm in intracranial EEG recorded from 17 patients with refractory epilepsy. For each of the resulting >1.6 million HFO detections, 33 features were computed, including duration, power, line length, etc. The topology of the feature space was assessed using a non-parametric estimate of intrinsic dimension, and changes in the topology relative to time and channel were quantified using angular distance and a generalized Grassman-chordal distance. Linear discriminant analysis was used per channel to reduce the dimension, followed by a support vector machine (SVM) to classify the HFOs. This process was used to classify whether each HFO had ictal features. We then limited analysis to interictal periods and used the rate of "ictal-like" HFOs to identify seizure onset zone. This method was more accurate than using the rate of all HFOs, as is traditionally done. This demonstrates a novel, effective method to classify abnormal HFOs, which can have significant impact in their translation as epilepsy biomarkers.

Disclosures: W.C. Stacey: None. **K.R. Moon:** None. **A.O. Hero:** None. **S.V. Gliske:** None.

Poster

408. Epilepsy: Human Studies I

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 408.13/M5

Topic: B.11. Epilepsy

Title: Interictal determination of the seizure onset zone and laterality of patients developing a cognitive task

Authors: *J. GONZALEZ-DAMIAN[1], P. E. SAUCEDO ALVARADO[2], M. MONTES DE OCA[2], A. L. VELASCO[2];
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Abstract: Objective To determine laterality and seizure onset zone, using High frequency Oscillations (HFO), of deep brain electroencephalographic recordings from resistant medial temporal lobe epilepsy patients that were developing an emotion recognition task. **Methodology** Transversal, observational and analytic study that includes the electroencephalographic recordings of patients (n = 8) in surgical protocol from the *Clínica de Epilepsia* of *Hospital General de México*, between the years of 2014 - 2016. All records were obtained from patients developing an emotion recognition task. Detection of HFO's was done using the short time energy method. The frequency spectrum was calculated for a time window of twenty seconds around each oscillation using the wavelet approximation. It was calculated the high frequency oscillations index (HFOI), the frequency and amplitude for each oscillation detected. The results were analyzed using descriptive statistics and one-way ANOVA. **Results** The number of HFO's is larger in the channels associated with the seizure onset zone ($p = 0.01$). The number of HFO's is significant different when compared between patients ($p = 0.05$). Average amplitude of HFO's was 100 mV. HFOI allow us to determine, in 83% of the cases, laterality and seizure onset zone as compared with the standard analysis. **Conclusions** HFOI could help to determine laterality and seizure onset zone in patients who develop a cognitive task. HFOI could help to reduce the electroencephalographic recording time of patients in surgical epilepsy protocol.

Disclosures: J. Gonzalez-Damian: None. **P.E. Saucedo Alvarado:** None. **M. Montes de Oca:** None. **A.L. Velasco:** None.

Poster

408. Epilepsy: Human Studies I

Location: Halls B-H

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Program#/Poster#: 408.14/M6

Topic: B.11. Epilepsy

Title: Selective changes in cb1 receptor functional coupling to g-proteins in patients with temporal lobe epilepsy

Authors: *F. CARMONA CRUZ[1], M. CUELLAR-HERRERA[2], M. ALONSO-VANEGAS[3], R. CINAR[4], L. ROCHA[1];
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Abstract: Several studies indicate the endocanabinoids are involved in seizure activity. However, there is no information concerning signal transduction mechanisms downstream of the CB1 receptors in the human epileptic brain. In the present study, the CB1 receptor agonist-induced activation of the receptor/G protein complex was studied by measuring [^{35}S]guanosine´-γ-thiotriphosphate ([^{35}S]GTPγS) binding. The quantification of [^{35}S]GTPγS binding and its stimulation by selective receptor agonists represent an excellent experimental strategy to estimate if CB1 receptors are able to activate the nucleotide exchange on G_i/G_o proteins in the hippocampus and temporal neocortex of patients with temporal lobe epilepsy. The cerebral tissue was obtained from 24 patients during the epilepsy surgery. Their values were compared with values obtained of 6 subjects (autopsies) who died by accident and without history of neurological disease. The agonist-stimulated [^{35}S]GTPγS binding was evaluated in cell membranes. [^{35}S]GTPγS concentration assays revealed that [^{35}S]GTPγS binding stimulation by (R)-(+)-WIN 55,212+2, a CB1 agonist, was higher indicating augmented efficacy (Emax) in temporal neocortex (43.7%, $p<0.05$) versus autopsies (19.4%). When the same assay was conducted in hippocampus, the mean Emax value was not significantly different to autopsies (20.6%) versus patients (39.8%), indicating absence of CB1 receptor-mediated functional response in this brain area. The statistical analysis did not reveal significant differences in potency (EC_{50}) values in both brain areas. In conclusion, our present data obtained from patients with temporal lobe epilepsy provide strong evidence that CB1-induced signal transduction mechanisms downstream of these receptors are augmented in temporal neocortex, an effect non evident in hippocampus.

Disclosures: F. Carmona Cruz: None. **M. Cuellar-Herrera:** None. **M. Alonso-Vanegas:** None. **R. Cinar:** None. **L. Rocha:** None.

Poster

408. Epilepsy: Human Studies I

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Program#/Poster#: 408.15/M7

Topic: B.11. Epilepsy

Support: The Japan Epilepsy Research Foundation

Title: Vagus nerve stimulation activates inhibitory neuronal network in human cerebral cortex

Authors: *T. MATSUO[1], K. KAWAI[2], K. USAMI[3];
[1]NTT Med. Ctr. Tokyo, Tokyo, Japan; [2]Neurosurg., Jichi medical university, Tochigi, Japan; [3]Neurosurg., Natl. Ctr. for Child Hlth. and Develop., Tokyo, Japan

Abstract: Introduction: Vagus nerve stimulation (VNS) is a surgical treatment for intractable epilepsy as a palliative therapy for of its less invasiveness and good seizure outcome. More than half patients can get 50% seizure reduction compared to preoperative seizure frequency. Although VNS is widely used for drug resistant epilepsy, little is known about therapeutic mechanism how electrical stimulation of the left cervical vagus nerve reduce the occurrence of epileptic seizure. Since epileptic seizure is caused by abnormal cortical activity, it can be presumed that there exit direct or indirect affection of VNS on the cerebral cortex. On the other hand, gamma coherence is thought to reflect the activity of inhibitory neuronal network. In this study, we recorded intracranial electroencephalography (iEEG) during VNS on/off period, and investigated the inter-cortical interaction.
Method: Four patients with intractable epilepsy who had already started VNS therapy participated in this study. iEEGs, direct recording of cortical activities, during VNS were recorded in acute or chronic phase. Two of 4 patients were performed bilateral craniotomy, whereas 2 patients were unilateral craniotomy. In total, data from 6 hemispheres were analyzed. Neuronal activities were recorded through grid arranged macro electrodes with a sampling rate of 2kHz, and recorded signals were analyzed offline. Eleven electrode sheets were placed on the surface of frontal or temporal lobe in both sides. Gamma coherence ranging 60 to 120 Hz between each electrode pair during VNS on/off period was calculated per electrode sheet.
Results: We found elevated gamma coherence in stimulating period compared to inter stimulating period. Averaged gamma coherence of stimulating period was significantly higher (0.21 ± 0.21) than inter stimulating period (0.19 ± 0.19) (paired t-test, $p = 0.03$). This trend was more prominent in right hemisphere (0.14 ± 0.09 and 0.13 ± 0.08; $p < 10^{-4}$).
Conclusion: Our results suggest a part of therapeutic mechanism of VNS that increased activity of inhibitory neuronal network elicited by VNS especially observed in right hemisphere consequently suppresses seizure occurrence.

Disclosures: T. Matsuo: None. **K. Kawai:** None. **K. Usami:** None.

Poster

409. Oligodendrocytes

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Program#/Poster#: 409.01/M8

Topic: B.12. Glial Mechanisms

Support: MH41865

Title: Loss of primary cilia in the oligodendrocyte lineage lineage in relation to myelination

Authors: A. SUBEDI, *J. L. FUCHS;
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Abstract: Primary cilia are non-motile, solitary sensory organelles present in most vertebrate cell types. Cilia-dependent signaling by sonic hedgehog contributes to proliferation, migration, and differentiation in the generation of oligodendrocytes. However, there are no reports of cilia in mature, myelinating oligodendrocytes. The present *in vivo* study systematically investigates the incidence of cilia in the mouse oligodendrocyte lineage during postnatal development, when myelination occurs. Primary cilia, identified by antibodies to adenylyl cyclase type 3 and Arl13b, were present in oligodendrocyte progenitor cells (OPCs), consistent with hedgehog-dependent generation of OPCs and oligodendrocytes. From P7 to P14, the percentage of $Olig2^{+}$ OPCs with a cilium decreased significantly, from 43% to 22% in cortex and from 36% to 15% in the corpus callosum. Mature, myelinating ligodendrocyte cells, identified by APC/CC1 expression, did not have cilia at any postnatal age, P4 through adultood. This observation suggests that cilia are lost in the lineage when cells gain the capacity to myelinate, and that oligodendrocytes are among the small minority of vertebrate cell types lacking primary cilia. Along with the loss of cilia, the centrosomal proteins γ-tubulin and pericentrin were both downregulated as oligodendrocytes gained APC/CC1. Myelin gene expression and timely myelination require enhanced Wnt/β-catenin signaling. Cilia can sequester β-catenin, and correspondingly, cilia loss should promote activity in the Wnt/β-catenin pathway. We propose that cilia loss in oligodendrocytes may promote the expression of myelin genes. Both OPCs and mature oligodendrocytes were Olig1+, but only those Olig1+ cells that lacked cilia co-expressed TCF3, a member TCF/LEF family which is associated with Wnt/β-catenin signaling. The results suggest that cells in the oligodendrocyte lineage lose primary cilia when becoming myelinating oligodendrocytes, and support the hypothesis that cilia loss serves to enhance the Wnt/β-catenin signaling required for the timely onset of myelination.

Disclosures: A. Subedi: None. **J.L. Fuchs:** None.

Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: BBSRC Case Studentship BB/L502236/1

Title: Regulation of oligodendrocyte lineage cell function by the RXRγ nuclear receptor

Authors: *L. DI CANIO[1], A. GUZMÁN DE LA FUENTE[1], G. J. WAYNE[2], R. J. M. FRANKLIN[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]GSK, Stevenage, United Kingdom

Abstract: Remyelination is an efficient process whereby oligodendrocyte progenitor cells (OPC) restore myelin by differentiating into oligodendrocytes (OLG). However, with increasing age in chronic demyelinating diseases such as multiple sclerosis, remyelination becomes less efficient, leaving axons vulnerable to degeneration. Understanding the process of OPC differentiation lies at the essence of developing endogenous therapeutic interventions by which remyelination can be enhanced.
We have identified RXRγ, a nuclear receptor (NR) able to regulate gene transcription, as an important positive regulator of OPC differentiation. RXRγ does so by forming heterodimers with other NRs and by further associating to co-regulators. The heterodimerisation of RXRγ to specific NRs appears to determine the stage of differentiation within the OLG lineage *in vitro*. In particular, Vitamin D Receptor, Peroxisome proliferator-activation receptor γ and Retinoic acid receptor β have been shown to be major binding partners, each one promoting a different and specific stage of OPC differentiation. However, RXRγ is able to heterodimerise with numerous other NRs as well as binding co-regulators to exert its function.
Using both Proximity Ligation Assay technology and a high-throughput screen of various NR agonists and antagonists, we are identifying the prevalent RXRγ binding partners in determining the OPC state. Binding partners with the strongest effect will be further explored in ageing OPCs as well as in an *ex vivo* remyelination paradigm. Concurrently, we are determining the genes controlled by RXRγ in the OLG lineage using ChIP-sequencing, which will shed light on the specific mechanism by which RXRγ controls progenitor cell fate.

Disclosures: **L. Di Canio:** 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; GSK. **A. Guzmán de la Fuente:** None. **G.J. Wayne:** A. Employment/Salary (full or part-time): GSK. **R.J.M. Franklin:** None.

Poster

409. Oligodendrocytes

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National Multiple Sclerosis Foundation Pilot Grant (PAR)

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NIH DK68096 (CJF)

Title: Comparing probes for zinc detection in developing and mature oligodendrocytes

Authors: *C. M. ELITT[1,2], K. PATEL[1], J. WANG[1], C. FAHRNI[3], P. ROSENBERG[1,2]; [1]Neurol., Boston Children's Hosp., Boston, MA; [2]F.M. Kirby Neurobio. Ctr. and Harvard Med. Sch., Boston, MA; [3]Sch. of Chem. and Biochem. and Petit Inst. for Bioengineering and Biosci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Zinc is abundant in the central nervous system and required for proper function of a variety of enzymes, proteins and transcription factors. Increases in free ionic zinc (Zn^{2+}) play a critical role in neuronal and oligodendrocyte (OL) injury. Little is known about mechanisms of zinc regulation in developing or mature OLs and there is growing interest in imaging free ionic zinc to better understand the role of zinc signaling in normal cell function and development, as well as in cell death. We have previously shown that concentrations of free zinc are downregulated as OLs mature using Chromis-1, a novel Zn(II)-selective ratiometric fluorescent probe optimized for two-photon excitation fluorescence microscopy (TPEM) (Elitt et al., *SfN abstract* 481.08, 2015). The objective of the current study was to determine if other commonly used zinc probes such as Zinpyr-1 were comparable in detecting free ionic zinc in OLs. Mixed glia cultures were isolated from postnatal day 2 (P2) rat forebrains and grown for 10-17 days. Developing OLs were separated from microglia and astrocytes using selective detachment and then plated onto coverslips. Cells were maintained in the presence of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) for 8 days prior to imaging. To produce mature OLs, PDGF and FGF were replaced by triiodothyronine (T3) and ciliary neurotrophic

factor (CNTF). Zinc imaging was performed using Zinpyr-1 (Cayman Chemical) or Chromis-1. Interestingly, baseline patterns of zinc localization were distinctly different with the two probes. Zinpyr-1 localized to discrete, focal structures within the cytoplasm while Chromis-1 was more uniformly detected in the entire cytoplasm with a finer pattern of punctate staining. Both probes appeared to be excluded from the nucleus. Previous studies in cell lines have suggested that Zinpyr-1 localizes to the Golgi (Burdette et al., 2001), while Chromis-1 is found in mitochondria, endoplasmic reticulum and endosomes. Further subcellular characterization in oligodendrocytes is necessary. It is important to identify the zinc pools labeled by each probe to select the optimal tool for specific indications. Future studies will compare the dynamic changes of these zinc probes under conditions of injury relevant to disorders and diseases targeting OLs.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: NS082203

Capes, Brazil

Title: Transcription factor regulation by mTOR during oligodendrocyte differentiation

Authors: *I. M. ORNELAS, S. WAHL, L. KHANDKER, L. E. MCLANE, T. L. WOOD; Rutgers Univ., Newark, NJ

Abstract: Oligodendrocytes (OL) are generated from precursor cells that give rise to committed oligodendrocyte progenitor cells (OPCs), divide and migrate throughout the CNS. Differentiation of oligodendrocyte progenitor cells into mature oligodendrocytes requires extensive changes in gene expression. Several transcription factors play a role in the control of OL differentiation and such factors can be intrinsically and extrinsically regulated. We have shown that mTOR is critical for OL differentiation in vitro and in vivo (Tyler et al, 2009, 2011; Wahl et al., 2014). Here we furthered that analysis by investigating how mTOR controls the transcription factor machinery that is essential for the OPCs progression through the OL lineage. In the spinal cord of mice lacking mTOR in the oligodendrocyte lineage (CNP-Cre, floxed-mTOR) we found a significant increase in the mRNA levels of the inhibitor of DNA binding-2 (Id2). Conditional

knockout mice show an accumulation in early progenitors, PDGFRα+ cells, at the expense of O4+ cells in spinal cord at PND10. Moreover, Id2 mRNA expression is increased in sorted PDGFRα+ cells. Id2 is a negative regulator of transcription and differentiation in the OL lineage and is mainly regulated by BMP/Smad pathway (Samanta and Kessler, 2004). OPC cultures differentiated in the presence of rapamycin showed increased levels of phospho Smad1/5/8 compared to untreated control cells. Upon mTOR inhibition *in vitro* and *in vivo* we also observed a decrease in total levels of Sip1, which is a negative regulator of the Smads. Our preliminary results suggest that mTOR modulates Id2 expression through a complex regulation of the transcriptional machinery at its promoter, which is essential for OPCs to differentiate into mature oligodendrocyte.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: National Multiple Sclerosis Society

Title: Regulation of oligodendrocyte progenitors through AMPA receptor signaling

Authors: *A. AGARWAL, L. CHAKRAVARTI, K. SPARKS, A. MENON, D. E. BERGLES; Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Oligodendrocyte precursor cells (OPCs) express Ca2+ permeable AMPA receptors and form synapses with unmyelinated axons throughout the CNS. In vitro studies suggest that AMPA receptor signaling in OPCs may influence their proliferation, migration and differentiation. The primary obstacle in evaluating the function of AMPA receptors in OPCs in vivo is that they express the same complement of receptors that are present in neurons, precluding pharmacological manipulations. To overcome this limitation, we developed two conditional knock-in mouse lines in which AMPA receptor signaling can be modified in a cell specific manner. One line allows inhibition of AMPA receptor-mediated Ca2+ influx through expression of EGFP-tagged GluA2 subunits of AMPA receptors (EGFP-GluA2) and another line inhibits the formation of functional AMPA receptors through expression of EGFP-tagged dominant negative GluA2 subunits (EGFP-dnGluA2). These constructs were targeted to the ROSA26 locus to ensure widespread expression in developing or adult tissue. To evaluate

whether EGFP-GluA2 and EGFP-dnGluA2 were expressed at levels sufficient to modulate AMPA receptor function in OPCs, we bred Rosa26-lsl-EGFP-GluA2 and R26-lsl-EGFP-dnGluA2 mice to NG2-CreER/+ mice. Double transgenic offspring (NG2-GluA2 and NG2-dnGluA2) were injected with 4-hydroxytamoxifen (4HT) at various developmental stages and were analyzed between 2-20 weeks post-injections. Analysis of OPC behavior in these lines indicate that blocking AMPA receptor function in OPCs in vivo enhances their survival and rate of proliferation during development, whereas reduces their differentiation into oligodendrocytes (OL). A detailed 3D analysis revealed that OPCs lacking AMPA receptor function are morphologically less complex with stubby processes. To investigate the role of this signaling in OPC recruitment following demyelination, we induced a unilateral focal demyelination in the corpus callosum of mutant and control mice by injecting 1.0% Lysolecithin; and mice were analyzed two weeks post-injection. The number of OPCs expressing EGFP-dnGluA2 was significantly enhanced in the demyelinated lesion. Subsequent genetic fate tracing experiments revealed that in lesions, blocking AMPA receptor function in OPCs enhances their potential to differentiate into OLs. Together, these selective manipulations of AMPA receptor signaling in OPCs indicate that this form of rapid neuron-glial signaling alters key aspects of OPCs behavior namely proliferation, and differentiation into OLs.

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Poster

409. Oligodendrocytes

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Support: NIMH

NINDS

Adelson Medical Research Foundation

ALS Association

Novo Nordisk Foundation

Title: *In vitro* pre-transplant patterning of forebrain and spinal cord oligodendrocyte fates from human pluripotent stem cells

Authors: *T. MAJOR[1,2], A. H. KUROPATNICKA[1], S. A. GOLDMAN[1,2];
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Abstract: Oligodendrocyte progenitor cells (OPCs) derived from human pluripotent stem cells (hPSCs) may be useful cellular reagents for the cell-based treatment of inherited and acquired disorders of central myelin. However, the extent to which regional patterning might limit the integration and/or function of hPSC-derived OPCs is unclear. To obviate this issue, we have established new protocols for specifically instructing OPCs to forebrain and spinal cord fates. Here we present protocols for the differentiation of forebrain (anterior) and spinal cord (posterior) OPCs from hPSCs, each based on a modified version of the dual-SMAD inhibition method. Both experimental approaches recapitulate the key region-specific developmental and transcriptional milestones of embryonic oligodendrocyte development. Combined application of LDN-193189, SB43154 and Pumorphamine with the small molecule inhibitor XAV939 or Retionic Acid results in efficient specification of forebrain (Pax6/FoxG1) or alternatively spinal cord (Pax6/HoxB4) neural precursors (NPCs). Further cultivation of NPCs in glial differentiation media (Wang et al., Cell Stem Cell, 2013) potentiates proliferation and differentiation of OPCs. Characterization of the developmental stages of anterior and posterior OPCs was achieved by immunocytochemistry. Subsequent validation of the cell phenotypes was performed by microaaray profiling and qPCR analysis. Global transcriptome comparison of anterior and posterior OPCs expression profile showed the robust induction of oligodendrocyte specific genes (OLIG2, NKX2.2, SOX10, PDGFRalfa) as well significant expression of myelin-related genes (MAG, MBP). Forebrain identity was confirmed by enrichment for FOXG1, OTX1, EMX2 while the spinal cord phenotype was determined by the significant enrichment for HOX gene family members. Fluorescence activated cell sorting (FACS) targeting the oligodendrocyte sulfatide recognized by mAb O4 permitted the purification of the resultant cell pre-patterned oligodendrocyte populations, which were capable of efficient in vivo myelination in immunodeficient shiverer x rag2$^{-/-}$ mice. In addition to providing a readily scalable source of anterior and posterior OPCs suitable for region-specific transplantation, these protocols provide new opportunities for the developmental analysis and comparison of forebrain and spinal cord oligodendrogenesis. In addition, such regionally specified anterior and posterior OPCs may prove useful in modeling those immune and metabolic demyelinating disorders that preferentially target either the forebrain or spinal cord white matter.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: BBSRC

IBBS

Title: Oligodendrocyte precursor cells (OPCs) dynamics and Kir4.1 profile in ageing brain

Authors: *I. CHACON DE LA ROCHA, A. D. RIVERA, A. M. BUTT;
Univ. of Portsmouth, Portsmouth, United Kingdom

Abstract: Oligodendrocyte precursor cells (OPCs) are abundant in the mammalian central nervous system (CNS) during development and they persist in adult CNS where they produce oligodendrocytes, the myelin forming cells, throughout life. A unique feature of OPCs is that they contact synapses and nodes of Ranvier and are able to respond to synaptic signals due to the expression of different ion channels and neurotransmitter receptors. It is believed that this signalling from neurons could regulate the dynamics of OPCs and this may have an important role in myelin formation and remyelination, which it is essential for rapid communication and cognitive function. The causes of cognitive decline in normal ageing are complex. However, changes in OPCs and myelin loss in ageing brain had been reported. Here, we used Microarray to profile changes in genes expressed in the adult 3 months old and 18 months old mouse optic nerve. We identified Kir4.1, an inward rectifying potassium channel highly expressed in OPCs, as one of the most upregulated glial transcript affected in ageing. We are currently investigating whether this is related to white matter changes in ageing brain.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: Fondazione Cariplo, grant n° 2014-1207 to DL

FISM 2013/R-1 project to MPA

Title: Identification of a microRNA regulating the maturation of oligodendroglial precursor cells and pathologically up-regulated in human multiple sclerosis

Authors: *D. LECCA[1], D. MARANGON[1], G. T. COPPOLINO[1], A. MENÉNDEZ MÉNDEZ[2], A. FINARDI[3], G. DALLA COSTA[3], R. FURLAN[3], M. P. ABBRACCHIO[1];
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Abstract: In the mature central nervous system (CNS), oligodendrocytes provide support and insulation to axons thanks to the production of a myelin sheath. During their maturation to myelinating cells, oligodendroglial precursors (OPCs) follow a very precise differentiation program, which is finely orchestrated by transcription factors, epigenetic factors and microRNAs (miRNAs), a class of small non-coding RNAs involved in post-transcriptional regulation. Any alterations in this program can potentially contribute to dysregulated myelination, impaired remyelination and neurodegenerative conditions, as it happens in multiple sclerosis (MS).
Here, we identify a developmentally regulated miRNA as a new actor of oligodendroglial maturation, that, in the mammalian CNS regulates the expression of myelin genes by simultaneously acting on several of its already validated targets. In cultured OPCs, over-expression of this miRNA by mimic treatment impairs while its inhibition with an antago-miR stimulates oligodendroglial maturation. Moreover, we show that miRNA levels are abnormally high in the cerebrospinal fluid of MS patients bearing active demyelinating lesions, suggesting that its pathological upregulation may contribute to MS development, at least in part by blockade of OPC differentiation leading to impaired repair of demyelinated lesions.
Further studies will be necessary to validate this miRNA as a possible biomarker for different stages of MS, providing a previously unrecognized venue for medical interventions.

Disclosures: **D. Lecca:** None. **D. Marangon:** None. **G.T. Coppolino:** None. **A. Menéndez Méndez:** None. **A. Finardi:** None. **G. Dalla Costa:** None. **R. Furlan:** None. **M.P. Abbracchio:** None.

Poster

409. Oligodendrocytes

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Norton Healthcare

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Title: Autophagy regulates myelin compaction in the final stages of CNS myelination

Authors: *A. N. BANKSTON[1,2], M. D. FORSTON[1,2,3], A. E. SMITH[1,2], R. M. HOWARD[1,2], S. R. WHITTEMORE[1,2];
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Abstract: Myelination is critical for fast action potential conduction in vertebrates, and lack of myelin causes severe neurological disorders. Development of myelinating oligodendrocytes (OL), the sole providers of central nervous system (CNS) myelin, from proliferating oligodendrocyte precursor cells (OPCs) requires migration to target axons and defined morphological changes prior to myelination. The mechanistic basis for tight control of process extension, axon ensheathment, myelin wrapping, and the removal of cytoplasm to form compact myelin remain largely unknown. Here, we present evidence that autophagy, the targeted isolation of cytoplasm and organelles by the double-membrane autophagosome for lysosomal degradation, regulates myelin formation in mature OL. A marked increase in autophagic activity coincides with OL differentiation, with OL processes having the greatest increase in autophagic flux. Multiple lines of evidence indicate that autophagosomes form in developing myelin sheathes before trafficking from myelin to the OL soma. Pharmacological autophagy inhibition blocked myelination, producing OL surrounded by many short processes. Conversely, autophagy stimulation enhanced myelination while reducing the overall number of OL processes. Mice with OL-specific genetic deletion of the essential autophagy gene Atg5 develop a rapid tremor and die around postnatal day 12. Further analysis revealed nearly complete loss of CNS OPCs and reduced myelination. Intriguingly, the surviving Atg5-null OL produced myelin wraps that failed to compact. Mass spectrometric analysis of autophagosomes purified from differentiating OL identified actin and 2',3'-cyclic-nucleotide 3'-phosphodiesterase, proteins shown by others to be involved in myelin compaction. These results implicate autophagy as a key regulator of OPC

survival initially and myelin compaction during the terminal stages of myelination. Autophagy may provide an attractive target to both promote OL survival and subsequent myelin repair after injury.

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Poster

409. Oligodendrocytes

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Support: National Institute of Mental Health (K01MH087845)

Ray P. Authement College of Sciences start up funds from the University of Louisiana at Lafayette (KMS)

Undergraduate Mini Research Grant-University of Louisiana at Lafayette (KMS)

Title: Fibroblast Growth Factor Receptor 1 in a mouse model of demyelination

Authors: *K. M. SMITH[1], H. M. TORRES[2], J. C. COLLETTE[2];
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Abstract: Fibroblast Growth Factor Receptor 1 (Fgfr1) is a receptor for various Fgf ligands, including Fgf2, and has been implicated in multiple roles of CNS development and maturation including hippocampal stem cell proliferation, cerebellar development, corpus callosum formation via genesis of glial midline structures, and oligodendrocyte development. Consistent with these findings, we observe the tgFgfr1-EGFP BAC transgenic line to be expressed in the CNS in regions that are congruent with previously published in situ hybridization studies including embryonic ventricular zone, hippocampus, midline glial cells, hypothalamus, and cerebellar Bergmann glia. We have also observe Fgfr1 expression in cell types that are consistent with the known roles of Fgfr1 in development including Hippocampal stem cells and DCX positive progenitors, pyramidal neurons of the Hippocampal CA region, a majority of astrocytes, and in Olig2+ oligodendrocytes and oligodendrocyte precursor cells. An important question that remains to be answered is whether this model for tracing Fgfr1 expression can be used to investigate changes in GFP levels that would match that of the endogenous FGFR1 promoter. Specifically, can manipulations known to increase or decrease FGFR1 mRNA expression be

detected as changes in GFP expression? The cuprizone model of demyelination is well-studied model for examining oligodendrocytes and their precursors during the process of remyelination. In the acute model of cuprizone demyelination, animals eating a chow with cuprizone (typically .2% cuprizone) for 6 weeks develop demyelination in the corpus callosum. Fgfr1 expression as detected by in situ hybridization has been reported to increase in the corpus callosum in this model. We have performed cuprizone demyelination studies in the tgFgfr1-EGFP BAC transgenic line, and will compare the GFP expression levels in the cortex, corpus callosum and hippocampus in cuprizone (.2%) treated animals and control chow treated animals. We will further examine whether upregulation occurs in oligodendroctyes, astrocytes, or both cell types. Future studies will also aim to examine changes associated with a chronic model of cuprizone demyelination.

Disclosures: K.M. Smith: None. **H.M. Torres:** None. **J.C. Collette:** None.

Poster

409. Oligodendrocytes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 409.11/M18

Topic: B.12. Glial Mechanisms

Support: Multiple Sclerosis Society of Canada

Canadian Institute of Health Research

Title: Investigating immune system activation following loss of the Quaking (QKI) RNA binding proteins using PLP-CreERT

Authors: *L. DARBELLI, S. RICHARD;
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Abstract: Objective: Recently, we established that the adult inducible loss of QUAKING (QKI) RNA binding proteins in myelinating cells using *PLP-CreERT* results in impaired mobility, thoracic kyphosis and death by 30 days post tamoxifen injection (Darbelli L et al, 2016, J. Neurosci 36:4106-20). Adult inducible removal of myelin genes does not normally lead to lethality with little to no immune system involvement. Therefore, we sought to investigate whether immune system activation might be contributing to the severity of the phenotype in QKI-deficient mice. **Methods:** C57BL/6 $QKI^{FL/FL;PLP\text{-}CreERT}$ inducible knockouts (iKO) and wild-type (WT) littermates were used in our study. Eight-week-old mice were injected with tamoxifen to induce recombination and monitored thereafter. We used Fluorescence activated cell sorting

(FACS) to assess immune system activation and T-cell infiltration in the spinal cord of mice. Immunostaining was used to assess microglia activation, macrophage and T cell infiltration. **Results:** FACS of microglia isolated from central nervous system (CNS) of WT and iKO mice has shown an increased MHC class II expression in the iKO mice indicating that those cells are activated. Moreover, quantitative RT-PCR has shown an increased expression of microglia activation markers including C1qA and CD68 in spinal cords of iKO compared to WT, as well as an increase in pro-inflammatory cytokines and chemokines such as IL-1β and Ccl2, respectively. FACS analysis has also shown a lack of double positive $CD4^+CD8^+$ T-cells in the thymus and an increase in $CD4^+$ and $CD8^+$ T-cells populations in the spleen, which we are currently investigating. **Conclusion:** Microglia represent the first line of defense in the CNS. Therefore, we conclude that there may be some tissue damage or myelin debris that activates the microglia. This in turn might lead to activation of adaptive immune system and recruitment of T-lymphocytes in the CNS that will exacerbate the damage and contribute to the observed lethality in iKO mice.

Disclosures: L. Darbelli: None. **S. Richard:** None.

Poster

409. Oligodendrocytes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 409.12/N1

Topic: B.12. Glial Mechanisms

Support: Research Grants Council, HKSAR Gov't Project No.: GRF 660813

Title: DNA damage-associated loss of cortical oligodendrocytes in dementia and Alzheimer's disease

Authors: *K.-H. TSE, A. CHENG, F. MA, H. CHOW, K. HERRUP;
The Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: The loss of myelin is a well-recognized, but poorly studied pathology of the aging brain. We have found loss of myelin, associated with a significant reduction in the number of oligodendrocytes (OL), occurs early in the brains of the R1.40 transgenic mouse model of Alzheimer's disease (AD). To extend these observations to humans with cognitive decline, we examined the prefrontal cortex of subjects with AD or plaque-free dementia (DEM) as well as normal controls (NC) (n = 8 for each, age = 72.3 [55-86], female = 50%). Using Black Gold II staining we observed fine myelinated fibers in the gray matter of frontal cortex in normal controls, but not in either AD or DEM subjects. The disappearance of gray matter myelin was

accompanied by a reduction in OL density (Olig2-positive, $p < 0.01$). Using myelin regulatory factor (MyRF) as a measure, we found that the number of actively myelinating OLs was reduced, in gray matter ($p < 0.0001$), but not in white matter. In DEM and AD brains, we found that Olig2+ cells had evidence of increased DNA damage (enhanced nuclear γH2A.X and 8-oxoguanine) and cell death (cleaved caspase 3). Gene expression profiling revealed an upregulation of genes related to DNA repair (ATM, ERCC1, ERCC2), and downregulation of genes associated with differentiated myelin genes (OLIG2, MBP and PMP22) in both DEM and AD group relative to NC. Further analysis revealed an inverse correlation between ATM and ERCC expression and Olig2+ and MyRF+ cell density in all subjects (Pearson r = -0.48 to -0.72, $p < 0.05$). To test the hypothesis that the DNA damage is a cause rather than a consequence of these events, we examined ataxia telangiectasia mutated knockout mice (*Atm*$^{-/-}$). As in human AD and the R1.40 mouse, the density of MyRF+ OLs was significantly reduced in cortical gray matter, but not in corpus callosum of 3 month-old *Atm*$^{-/-}$ animals; myelin gene expression (*Mbp, Mag, Myrf,* and *Ng2*) was downregulated as early as 1 month of age. Intracortical myelin degeneration is an early pathology in the ageing brain that likely contributes to cognitive decline. The preferential loss of cortical OL in the gray matter, and its strong association with DNA damage, suggests that unrepaired genetic damage drives myelin loss in dementia. The similarity of our findings in AD and DEM suggests that myelin pathology in dementia is likely caused by amyloid-independent mechanisms.

Disclosures: K. Tse: None. **A. Cheng:** None. **F. Ma:** None. **H. Chow:** None. **K. Herrup:** None.

Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: Eranet Neuron project RENEW-IT to ET and MPA

Fondazione Cariplo Rif. 2015-0910 to MF

Piano di Sostegno alla Ricerca 2015-17, Linea 2 to MF

Title: GPR17-expressing oligodendrocyte progenitors participate in the reparative response after brain ischemia and their behaviour is influenced by microglia-derived vesicles

Authors: *M. FUMAGALLI[1], E. BONFANTI[1], P. GELOSA[2], M. LOMBARDI[3], E. TREMOLI[2], M. CIMINO[4], L. DIMOU[5], L. SIRONI[1], C. VERDERIO[6], M. P. ABBRACCHIO[1]; [1]Dept. of Pharmachological and Biomolecular Sci., Univ. Degli Studi Di Milano, Milano, Italy; [2]Ctr. Cardiologico Monzino IRCCS, Milan, Italy; [3]IRCCS Humanitas, Rozzano, Mi, Italy; [4]Univ. of Urbino, Urbino, Italy; [5]Physiological Genomics, Biomed. Center, Ludwig-Maximilians Univ., Munich, Germany; [6]CNR Inst. of Neurosci., Milan, Italy

Abstract: It is currently acknowledged that an ideal therapeutic intervention to decrease stroke-associated disability should comprise both neuroprotective and neurorestorative approaches aimed at implementing local spontaneous post-injury repair mechanisms (Zhang and Chopp, 2009 Lancet Neurol).
Recent data obtained by fate-mapping analysis using the conditional GPR17-iCreERT2xCAG-eGFP transgenic mice, showed that the subpopulation of adult oligodendrocyte progenitors (OPCs, also known as NG2-glia) expressing the GPR17 receptor represents "a reserve pool" of OPCs, that is maintained for repair purposes after brain damage, including ischemic stroke (Viganò et al., 2016 Glia).
Here, we aimed at i) providing a detailed analysis of the fate and behavior of cells expressing GPR17 at different times after Middle Cerebral Artery occlusion (MCAo) in GPR17-iCreERT2xCAG-eGFP mice, and ii) exploring the cross-talk between microglia, the CNS resident immune cells, and OPCs, by assessing how vesicles released extracellularly by microglia (EVs) influence OPC behavior.
Starting from 72h after MCAo, we observed that GPR17 expressing-OPCs actively respond to the ischemic insult by increasing their capability to proliferate. At the same time point, cells in the dorsal cortex regions close to the ischemic area are polarized and display more directional branch extension toward the site of damage, suggesting OPC recruitment toward the lesion area. Data collected at 8 weeks after MCAo showed that some recombined cells at the border of the ischemic lesion also stain for the mature marker GSTpi, indicating that only a fraction of cells, belonging to the GPR17-positive pool of NG2 glia, has differentiated into mature oligodendrocytes.
Of note, *in vitro* studies pointed out that EVs produced by M1 pro-inflammatory microglia hinder OPC proliferation. On the contrary, 48h exposure to EVs from either M1 or M2 pro-regenerative microglia (but not resting cells) promotes OPC maturation, with EVs from M2 cells displaying higher activity. Interestingly, this is accompanied by GPR17 downregulation. These data suggest that microglial derived EVs may contain signals able to influence OPC proliferation and trigger their terminal maturation. Thus, shedding light on the mechanisms by which microglia activation interferes with the regeneration potential of OPCs is important for developing effective therapeutic interventions to implement functional recovery after ischemic stroke.

Disclosures: M. Fumagalli: None. **E. Bonfanti:** None. **P. Gelosa:** None. **M. Lombardi:** None. **E. Tremoli:** None. **M. Cimino:** None. **L. Dimou:** None. **L. Sironi:** None. **C. Verderio:** None. **M.P. Abbracchio:** None.

Poster

409. Oligodendrocytes

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Program#/Poster#: 409.14/N3

Topic: B.12. Glial Mechanisms

Title: Glial disruptions in response to cerebral hypoperfusion in young adult and aged rats

Authors: E. T. CURFMAN[1], H. C. PARAISO[2], *R. D. SWEAZEY[3], F.-L. CHANG[1], I.-C. YU[1];
[1]Indiana Univ. Sch. of Medicine-Fort Wayne, Fort Wayne, IN; [2]Indiana University-Purdue Univ. Fort Wayne, Fort Wayne, IN; [3]Indiana Univ. Syst., Fort Wayne, IN

Abstract: Vascular dementia (VaD) accounts for up to 20% of age-related dementia and presents a significant care burden on elderly patients and their caregivers. This form of cognitive impairment results from cerebral hypoperfusion and presents as diffuse white matter abnormalities that increase in severity with age. Given the myelinating activity of oligodendrocytes and their susceptibility to oxidative stress, the importance of glial mediation between vascular dysfunction and cognitive impairment is increasingly being recognized. Glial cells respond uniquely to disruptions in cerebral blood flow (CBF) based on their cell-type and location within a hypoperfused region, and deep white matter tracts are particularly vulnerable to ischemic insults. One model for VaD in rats is produced by permanent bilateral common carotid artery occlusion (BCCAO), and previous research has examined its effects in 3 month old animals. Young animal studies are common and exhibit up to 70% reductions in CBF for 3 days post-occlusion. However, few studies have examined aged animals. Given that the clinical presentation of VaD is primarily in elderly patients, and that white matter abnormalities increase in severity with age, studies using aged rats are warranted. To address this, we investigated VaD in both young adult (4-6 month old) and aged (24 month old) SD rats after BCCAO. We present findings that even in cases where CBF compensates rapidly after BCCAO, glial cells undergo long-term changes in both young adult and aged groups. These changes include reduction of mature oligodendrocyte mRNA markers CNPase, myelin basic protein, and myelin associated glycoprotein at 2 weeks post-BCCAO. At 4 weeks post-BCCAO, significant disruptions in white matter tracts can be seen by luxol fast blue staining in both young adult and aged groups. Furthermore, increases in GFAP stain intensity between young adult and aged rats indicate age-related changes in the astrocyte population. In adult animals, an increase in GFAP-positive cells in the superior medial striatum adjacent to the lateral ventricle indicates region-specific astrocytic response to cerebral hypoperfusion. We conclude that BCCAO produces multiple disruptions of glial cell functions following cerebral hypoperfusion in aged animals.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: AHA Predoctoral Fellowship 15PRE22580000

AHA/Bugher Foundation 14BFSC17760005

Title: A stroke-specific oligodendrocyte progenitor cell transcriptome reveals novel genes impacting recovery after white matter stroke

Authors: ***D. J. DITULLIO**, E. G. SOZMEN, S. T. CARMICHAEL;
Interdepartmental Program for Neurosci., UCLA, Los Angeles, CA

Abstract: Stroke is the leading cause of adult disability. White matter stroke (WMS) comprises 25% of all stroke, yet no treatments exist to address its devastating effects. Death of oligodendrocytes and subsequent demyelination is a major pathologic mechanism in WMS, and normal regeneration of oligodendrocytes by their progenitor population (OPCs) is inhibited. This differentiation block is the primary focus of the current study, as increasing myelination by oligodendrocytes after WMS has been linked to functional improvement.

We have developed a stroke-specific OPC gene transcriptome that provides insight into potential mechanisms underlying the WMS-specific OPC differentiation block. OPCs were isolated from the peri-infarct white matter using laser capture microdissection at two time points following injury: 5 days after stroke at the peak of the proliferative response, and at 15 days after stroke, at the peak of differentiation. Rigorous bioinformatics analysis of the OPC stroke transcriptome using a combination of statistical significance values, fold change in expression, and relative expression intensity measures has led to the generation of a quantitative priority list of ten genes predicted to most significantly impact OPC differentiation.

These ten genes are the subject of the current studies. Each gene is investigated first using primary OPC cultures as a screening system. Each gene of interest is up- and down-regulated in isolation, and effect on OPC differentiation is analyzed using qPCR and morphology analysis. Using results from these primary OPC culture studies, genes have been prioritized by their propensity to promote OPC differentiation. The two genes that most significantly influence OPC differentiation are being manipulated in vivo in a mouse model of WMS. Overexpression of each

gene via lentiviral induction allows for direct analysis of OPC response in the peri-infarct region. Fate analysis and myelin basic protein expression levels in the corpus callosum are used to analyze white matter recovery after WMS in control and test animals. Follow-up studies will focus on uncovering the mechanisms by which these genes play a role in OPC differentiation and remyelination after white matter stroke, improving our understanding of the unique pathologies of white matter ischemia and uncovering new potential targets for treatment.

Disclosures: D.J. DiTullio: None. **E.G. Sozmen:** None. **S.T. Carmichael:** None.

Poster

409. Oligodendrocytes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 409.16/N5

Topic: B.12. Glial Mechanisms

Title: Functional heterogeneity of oligodendrocyte progenitors in the central nervous system

Authors: *S. FÖRSTER[1], A. C. CRAWFORD[1], R. B. TRIPATHI[2], W. D. RICHARDSON[2], R. J. M. FRANKLIN[1];
[1]Clin. Neurosci., Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. Col. London, London, United Kingdom

Abstract: In the mouse embryonic forebrain oligodendrocyte progenitors (OPs) are generated in consecutive waves from distinct brain regions along a spatiotemporal gradient, with ventral OPs emerging before dorsal OPs. Although these different OP populations functionally compensate during development, they persist in the brain throughout life. To investigate whether ventrally and dorsally derived OPs fulfil different functions in the adult brain, dorsally derived OPs were ablated using a Sox10-driven diphtheria toxin A (DTA) mouse model. As dorsally derived OPs mainly populate the cerebral cortex, locomotor coordination after the ablation of dorsally derived OPs was assessed first. Mice ablated of dorsally derived OPs show significant impairment in locomotor coordination. These impairments are not due to a decrease in overall brain white matter content shown by magnetic resonance imaging. Assessing the differentiation capabilities of ventrally and dorsally derived oligodendrocyte lineage cells *in vivo* demonstrated that after an initial delay in differentiation of dorsally derived OPs early after birth, ventrally and dorsally derived OPs give rise to similar numbers of mature oligodendrocytes in the adult brain. Our results point towards a distinct role of ventrally and dorsally derived OPC in executing specific brain functions in the adult brain. A more detailed analysis of signalling pathways differentially regulated in ventrally and dorsally derived OPs, and myelin structures formed by

oligodendrocytes derived from the distinct OP populations will help to further uncover the underlying difference of the two developmentally distinct OP populations.

Disclosures: **S. Förster:** None. **A.C. Crawford:** None. **R.B. Tripathi:** None. **W.D. Richardson:** None. **R.J.M. Franklin:** None.

Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: National Multiple Sclerosis Society RG5274A1/T

WSU Special Projects Grant

Title: Preferential axon-oligodendrocyte interactions at axon varicosities preceding initial myelin ensheathment

Authors: ***M. MARTELL**, B. B. DUXBURY, S. W. STEELE, A. G. TRUDEL, A. J. TREICHEL, J. H. HINES;
Winona State Univ., Winona, MN

Abstract: Oligodendrocytes, the myelinating cell type of the central nervous system, extend exploratory membranous processes that sample numerous axons before beginning the initial wrapping of axons. During this process, specific axons will be chosen for myelination while other axons will never be wrapped. Although the functions and importance of myelin have been well characterized, the mechanisms directing oligodendrocytes to initiate myelin ensheathment are poorly understood. The objective of this study is to determine how oligodendrocytes recognize and distinguish specific axonal types prior to initial wrapping. One possibility is that oligodendrocytes select specific axons based on axon diameter, whereby axons with higher caliber are preferentially selected. To examine the axon-glia interactions preceding myelination, we performed in vivo imaging using transgenic zebrafish reporters that mark myelin-fated axons and oligodendrocytes. Both before and during initial ensheathment, we observed significant variability in thickness along the length of single axons. In vivo time-lapse imaging showed that axon morphology is highly dynamic before and during initial wrapping. Axons possessed swelling points, also known as varicosities, that underwent brief and localized radial growth. A subset of axon varicosities were transient, turning over within seconds, while others remained stable for greater than two hours of time-lapse imaging. Importantly, varicosities showed

frequent and sustained co-localization with pre-myelinating oligodendrocyte membrane processes. Prior to initial axon wrapping, axon varicosities interacted with oligodendrocyte processes 89.9% of the time, whereas neighboring thin axon segments showed interactions only 13.5% of the time. After initial wrapping, local axon diameter was greater at sites of new myelin ensheathment, suggesting that efforts to myelinate or stabilize myelin sheaths may be influenced by axon varicosities. Altogether, our findings demonstrate new cellular interactions between axons and oligodendrocytes that may specify axonal domains for ensheathment and stabilize wrapping attempts to facilitate myelination.

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Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: PhD Scholarship from Anatomical Society UK

Title: The role of neuronal activity in myelination, axon targeting and maintenance of specified cortical projection neuron populations

Authors: *K. KORRELL, A. HOERDER-SUABEDISSEN, Z. MOLNÁR;
Oxford Univ., Oxford, United Kingdom

Abstract: Myelin in the central nervous system is produced by oligodendrocytes to ensure fast saltatory conduction of action potentials. Myelination is dependent on neuronal activity but the underlying mechanism is not yet completely understood. Because myelination has been implicated in many neuronal diseases (multiple sclerosis, schizophrenia) it is important to understand the underlying basics. To do so, neurotransmission will be suppressed in selected populations of cortical Layer V and VI projection neurons to determine how neuronal activity influences myelination. The fusion of neurotransmitter containing vesicles is mediated amongst others by the SNAP25 protein. To inhibit regulated neurotransmitter release Nrsr1 (for Layer VI) and Rbp4 (for Layer V) conditional Snap25 KO mice are bred on a floxed-stop-tdTomato background such that all silenced cells are tdTomato-labelled. The onset of myelination was established by assessing immunoreactivity for myelin basic protein and directed the period of investigation to the end of the first postnatal week and later. After the onset of myelination was determined, it was examined whether ‘silenced’ layer VI fibres in the brain develop myelin

according to the same pattern seen in control brains. Additionally, 'silenced' layer V and layer VI projections have been investigated at various adult ages, and an unexpected degeneration in silenced projections was observed. Transmission electron microscopic examination of corticospinal projections from 'silenced' layer V projections in spinal cord revealed that myelin does not form a compact layer but increasingly loose structure with multiple vacuoles. In the next step, myelination will be assessed when neuronal activity is altered by supressing the conduction of action potentials by introducing an additional potassium channel at the nodes of Ranvier via *in utero* electroporation. In summary, preventing neurotransmitter release in this mice did not affect the onset of myelination or fibre targeting but myelin membrane compaction as well as fibre and myelin maintenance.

Disclosures: K. Korrell: None. **A. Hoerder-Suabedissen:** None. **Z. Molnár:** None.

Poster

409. Oligodendrocytes

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Topic: B.12. Glial Mechanisms

Support: NIH Grant NS25304

NMSS Grant PP-1505-04265

Title: Proteolipid protein null mice exhibit altered numbers of oligodendrocytes

Authors: *E. A. GOULD[1], D. SHEPHERD[2], D. RESTREPO[1], W. MACKLIN[1];
[1]Univ. of Colorado Denver Sch. of Med., Aurora, CO; [2]Univ. of Colorado Denver, Aurora, CO

Abstract: Myelin is required for proper nerve conduction and normal axonal function. However, little is known about the impact of myelin that is present but dysfunctional. Proteolipid protein (PLP) is major constituent of myelin and is abundantly expressed by oligodendrocytes (OL). PLP knockout mice (PLP-null) generate myelin but exhibit progressive myelin dysfunction and eventual axonal degeneration. We determined that 6 month PLP-null mice had a dramatic increase in OL density by quantifying OL in cleared brain tissue with light sheet imaging. OL were labeled using a transgenic line that drives eGFP expression under the PLP promoter. By underclearing the samples, we were able to retain eGFP expression in the OL cytoplasm and quantify OL number. Histological examination of OL lineage markers in brain sections confirmed our results. Starting at 2 months of age, PLP-null mice showed a region-specific increase in OL density, specifically in the corpus callosum, striatum, olfactory bulb. However,

motor cortex and hippocampus OL density was unaltered. At 4 months and 6 months, OL density continued to increase in PLP-null mice overall, indicating a pathological recruitment of OL. Consistent with this hypothesis, labeling of proliferative events with EdU showed an increase in the number of newly generated OL in the olfactory bulb and motor cortex 3 weeks after EdU injection. In the olfactory bulb, the number of EdU labeled cells that were not OL lineage cells also increased. To determine the source of the EdU labeled cells we labeled with a 4 hour pulse of EdU and observed local proliferative events. Intriguingly, the number of cells proliferating locally in the olfactory bulb and motor cortex was unaltered, while the number of proliferating cells in the subventricular zone was increased in PLP-null mice. This data indicates that PLP-null mice exhibit a pathological increase in oligodendrogenesis emanating from the subventricular zone, leading to an increase in OL density in specific brain regions. Ongoing investigation aims at characterizing the pathophysiological process that results in altered OL numbers in PLP-null mice.

Disclosures: E.A. Gould: None. **D. Shepherd:** None. **D. Restrepo:** None. **W. Macklin:** None.

Poster

409. Oligodendrocytes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 409.20/N9

Topic: B.05. Transporters

Support: DFG CNMPB

Title: Oligodendrocytes labelling with Sulforhodamine 101 depends on astrocytic uptake via the thyroid hormone transporter OATP1c1 (slco1c1)

Authors: *S. HÜLSMANN[1,2], L. HAGOS[2];
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Abstract: The specific visualization of glial cells with fluorescence markers is an important issue in modern neurophysiology. To label astrocytes, the dye sulforhodamine 101 (SR101) is widely used. Here we investigated whether SR101 can be used for in vitro staining of oligodendrocytes in the CA1 region of the hippocampus and whether the known astrocytic transporter OATP1C1 (slco1c1) is required also for oligodendrocyte labeling. We used transgenic mice that expressed the green fluorescent protein under the control of the oligodendrocyte specific proteolipid protein (PLP) promotor, and found that oligodendrocytes indeed are labeled by SR101 in hippocampal slice preparations. After application of the OATP1C1-substrate L-Thyroxin a significant reduction of the oligodendrocyte staining was

observed. Since slco1c1 is not expressed in oligodendrocytes, we conclude that oligodendrocyte-labeling with SR101 requires SR101-uptake by astrocytes, which then diffuses to oligodendrocytes via heterotypic gap junctions of the pan-glial network. In summary, unequivocal identification of a particular cell type is not possible by SR101 only, and thus, extra caution is recommended when using SR101 in the future.

Disclosures: S. Hülsmann: None. **L. Hagos:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.01/N10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cedars-Sinai Medical Center Grant 223343

NIA Grant R01 AG15819

NIA Grant P30 AG10124

NIA Grant P30 AG10161

Title: A dual incretin receptor agonist is especially potent in reducing insulin resistance in brains of mild cognitive impairment (MCI) and Alzheimer's disease dementia (ADd) cases

Authors: *K. TALBOT[1], J. KVASIC[2], A. STUCKY[2], S. M. SHAH[2], K.-C. LEE[2], K. P. BAKSHI[2], M. CHATTOPADHYAY[2], A. KHAN[2], P. L. MCCLEAN[3], C. HOLSCHER[4], A. J. SAMOYEDNY[1], J. Q. TROJANOWSKI[5], R. WILSON[6], D. A. BENNETT[6], R. D. DIMARCHI[7], H.-Y. WANG[2];
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Abstract: Introduction. While the FDA has approved several drugs for relief of AD symptoms, they do not meet criteria for clinical effectiveness nor for substantial slowing of disease progression. Many scientists are thus calling for testing drugs targeting novel causal factors in AD, among the most promising of which is *brain insulin resistance*, which can cause or promote many AD pathologies and symptoms and which we showed to be a common and prominent feature of AD even in the absence of diabetes (Talbot et al., *J. Clin. Invest.* 122: 1316-1338,

2012). In brains from the APP/PS1 mouse model of AD, MCI cases, and AD dementia (ADd) cases, we have accordingly tested highly potent antidiabetics for their ability to reduce brain insulin resistance. We specifically tested incretin receptor agonists (IRAs), which are agonists of glucagon-like peptide 1 (GLP-1) and/or gastric inhibitory peptide (GIP) receptors. Methods. An ex vivo stimulation method (Talbot et al., 2012; Wang, H.-Y. J. Neurosci. 9: 9773-9784, 2012) was used to quantify responses of the hippocampal formation (HF) from APP/PS1 mice, non-amnestic MCI (naMCI), amnestic MCI (aMCI), and AD dementia (ADd) cases to 30 min incubation in 1 or 10 nM insulin compared to controls. The human tissue was obtained from cases within 10 h of death. We quantified insulin-induced activation of the insulin receptor, insulin receptor substrate-1, and Akt. IRA effects on HF insulin responses were tested in mice 2 mo after daily 25 nmoles/kg body wt. injections (ip) of the GLP-1R agonist liraglututide (Victoza) and in humans 30-60 min after exposure of the HF to liraglutide (100 nM), a GIP receptor agonist (100 nM), or the dual GLP1/GIP receptor agonist (RO6811135, 100 nM). Results. In the HF from APP/PS1 mice, ip liraglutude treatment virtually abolished the profound insulin resistance in that brain area by 7 mo. of age. In the HF from MCI cases, all 3 IRAs reduced insulin resistance markedly ($p < 0.001$), but the dual IRA was superior to other IRAs in aMCI. In the HF of mid-to-late stage ADd cases, liraglutide and a GIPR agonist reduced insulin resistance to a small but significant degree ($p < 0.05$). Only the dual IRA, however, markedly reduced HF insulin resistance in the ADd cases ($p < 0.001$). Conclusions. Incretin receptor agonists significantly decreased HF insulin resistance in APP/PS1 mice, MCI cases, and ADd cases. In acute tests on human brain tissue, the most potent of these agonists was a dual GLP1/GIPR agonist, which was the only IRA that substantially reduced brain insulin resistance in ADd. Given the apparent linkage between brain insulin resistance and AD pathology and cognitive deficits, this dual IRA may prove to be an effective AD therapeutic.

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Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.02/N11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Stevenage Bioscience Catalyst Open Innovation Challenge Grant

Title: Unexpected retinal structure and changes in people with Down's syndrome, a high risk population for Alzheimer's disease

Authors: ***M. J. WALPERT**[1], E. M. NORMANDO[3], S. H. ZAMAN[2], F. M. CORDEIRO[3], A. J. HOLLAND[2];
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Abstract: Retinal biomarkers have the potential to provide insight into the natural progression of Alzheimer's Disease (AD). Early markers of AD-related change are necessary to aid the development and testing of treatments and therapies. It is understood that an increased knowledge of AD in Down's syndrome will benefit AD in the general population, due to the similarities in the manifestation of the disease. The aim of this project is to examine the potential of retinal degeneration as an early biomarker for AD in adults with DS. People with DS have a high prevalence of early onset AD. Spectral-domain optical coherence tomography (SD-OCT) was used to assess the structure of the DS retina and to detect retinal nerve fibre layer (RNFL) thickness. Fifty-four participants with DS and 68 healthy controls underwent high-resolution OCT examinations of RNFL. Cognitive ability and a diagnosis of dementia was assessed in participants with DS and control participants were screened for dementia. We anticipated that the RNFL would be significantly thinner in participants with DS than healthy controls based on dementia research which strongly indicates a decline in RNFL thickness with severity of the disease. RNFL is also known to naturally decrease in thickness in normal ageing. However, our results showed that RNFL is significantly thicker, rather than thinner, in people with DS (controls mean =97.34μ (SD =8.07μ), DS mean 118.46μ (SD =11.68), p =<0.001). Additionally we found that the DS RNFL measurements do not decline with age as seen in normal ageing and in the dementia population (DS r = -0.062, p =0.671). We suggest that the increased retinal thickness may be caused by deposition of amyloid-beta (Aβ) plaques in the retina, the toxicity of which may in turn increase the rate of apoptotic cells in the retina. Apoptotic cell bodies may build up in the retina due to impaired phagocytosis in DS causing increased thickening. A new study is now underway to measure rates of apoptosis using a novel technique, Detection of Apoptosing Retinal Cells (DARC), which has the capability of visualising programmed cell death in individual cells *in-vivo* for the first time.

Disclosures: **M.J. Walpert:** None. **E.M. Normando:** None. **S.H. Zaman:** None. **F.M. Cordeiro:** None. **A.J. Holland:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.03/N12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Combination dosing of the novel M1/M4-selective muscarinic agonist NSX-0527 and peripheral muscarinic antagonists for the treatment of Alzheimer's disease

Authors: S. A. HANSON[1], J. C. OCKULY[1], J. D. BECK[1], *M. L. HENDRICKSON[2,1]; [1]NeuroSolis, Inc., Madison, WI; [2]BrainXell, Inc., Madison, WI

Abstract: Although muscarinic agonists have long held promise for CNS disorders such as Alzheimer's disease (AD), development has been stymied by poor central potency and the potential for muscarinic side effects at high doses. For example, the M1/M4-preferring agonist xanomeline demonstrated efficacy in AD patients in a Phase II trial, but was abandoned due to poor tolerability. More recently, the novel M1/M4-selective orthosteric muscarinic agonist NSX-0527 (and close-in analogue NSX-0559) has been developed. NSX-0527 shows good bioavailability (~75%) and brain penetration (~60%) along with excellent metabolic stability. A combination therapy including FDA-approved peripheral muscarinic antagonists was evaluated in animal models as an approach to improve tolerability of high oral doses of NSX-0527. The peripheral antagonists evaluated, darifenacin, glycopyrrolate, methscopolamine, propantheline, and trospium, have extremely low brain penetration, resulting in an absence of side effects while not impeding the CNS activity of NSX-0527. Thus, such fixed-dose combinations may allow for increased agonist doses to aggressively drive central muscarinic M1 and M4 activation, and would represent a potential first-in-class therapeutic for AD.

Disclosures: S.A. Hanson: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **J.C. Ockuly:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **J.D. Beck:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **M.L. Hendrickson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc..

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.04/N13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Magceutics Inc.

Title: Open label trial of Magnesium l-Threonate for adults with mild to moderate Alzheimer's disease

Authors: *N. L. RASGON[1], T. WROOLIE[1], K. WATSON LIN[2], A. KRAMER[3], D. BALZAFIORE[1];
[2]Psychiatry and Behavioral Sci., [1]Stanford Univ. Sch. of Med., Palo Alto, CA; [3]Palo Alto Univ., Palo Alto, CA

Abstract: In the United States, there are an estimated 5.2 million cases of Alzhiemer's Disease (AD), with AD and other dementias effecting nearly 1 in 3 senior adults. Emerging research on Magnesium l-Threonate (MGT), provides evidence that supplementation may be of benefit to people with AD. Preliminary human trials and animal experiments have found associations between oral MGT supplementation and cognitive improvement, as well as neurobiological changes, including upregulation of NMDAR signaling pathways. We conducted an open-label trail of MGT supplementation of 17 adults aged 60 and older with mild to moderate AD (MMSE 14-26). The study consisted of 2 months of MGT supplementation followed by a 4 month follow up period. All subjects were given a complete neurocognitive battery at baseline, 2 months and 6 months. 18-F FDG PET scans were conducted at the study's baseline and after 2 months of treatment. Our results indicate a significant improvement in MMSE score from baseline to 2 months (mean change = 1.57 points SD=.67, p=.035), and between baseline and 6 months (mean change = 1.21 points SD=.60, p=.062). There was no significant change between 2 and 6 months (4 months after treatment discontinuation). The RBANS attention index, subtest for semantic fluency, and coding subtest each indicated a maintenance of cognitive function from baseline through 2 months and a significant decline after discontinuation. 18F FDG-PET scan showed a significant increase in regional cerebral metabolism in brain regions between baseline and two months follow up, including the medial temporal region, prefrontal cortex, parietotemporal cortex, and posterior cingulate cortex. In summary, while existing research MGT supplementation provides a preliminary evidence for its benefits to in the treatment of AD, relatively little research has been conducted overall, particularly in humans. Given the plausible mechanism of action and evidence of the effects of treatment on cognition and regional cerebral brain metabolism, additional human clinical trials are warranted.

Disclosures: **N.L. Rasgon:** None. **T. Wroolie:** None. **K. Watson Lin:** None. **A. Kramer:** None. **D. Balzafiore:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.05/N14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SUVN-502, a pure 5-ht6 receptor antagonist - proof-of-concept study design in moderate alzheimer's disease patients

Authors: ***R. V. NIROGI**, K. MUDIGONDA, K. PENTA, G. BHYRAPUNENI, V. BENADE, N. MUDDANA, V. PALACHARLA, D. AJJALA, V. GOYAL, S. PANDEY, R. ABRAHAM, R. KAMBHAMPATI, T. BANDYALA, V. BHATTA, A. SHINDE;
Suven Life Sci., Hyderabad, India

Abstract: SUVN-502 was evaluated in healthy human subjects following single and repeated administration. SUVN-502 was well tolerated following single or multiple oral administrations. There were no clinically relevant or serious adverse events. SUVN-502 is a pure 5-HT_6 receptor antagonist and demonstrated robust efficacy in preclinical animal models. The effect of SUVN-502 was studied in combination with memantine and donepezil in object recognition task using rats. SUVN-502 was also evaluated for the modulation of neurochemical (brain extracellular acetylcholine using microdialysis) and electrophysiological properties (theta and gamma modulation) in combination with donepezil and memantine. The exposures required for efficacy was projected based on the concentrations of SUVN-502 and its active metabolite M1 of SUVN-502 in animal models. Co-treatment of SUVN-502 with memantine and donepezil significantly potentiated the procognitive effects when compared to memantine and donepezil treatment group. These effects were seen even after repeated treatments for 14 days. SUVN-502 potentiated the effects of donepezil and memantine in hippocampal acetylcholine levels and brain oscillatory activity. There were no significant changes in the exposures of SUVN-502 or donepezil or memantine in the co-treatment group. The enhanced procognitive effects seen in the group co-treated with SUVN-502, memantine and donepezil can be attributed to the augmentation of the cholinergic neurotransmission in the brain. Thus, combination of SUVN-502 with memantine and donepezil may offer a new therapeutic strategy for the symptomatic treatment of Alzheimer's disease. SUVN-502 and M1 of SUVN-502 achieved the projected efficacy concentrations and attained steady state on day 7 upon multiple administrations in elderly human subjects. SUVN-502 has favorable pharmacokinetic profile suitable for once a

day oral administration. SUVN-502 is being evaluated in moderate AD patients currently treated with donepezil and memantine. A total of 537 subjects aged between 50 to 85 years are being enrolled for phase 2 study in USA.

Disclosures: **R.V. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Mudigonda:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Penta:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **N. Muddana:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Palacharla:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **D. Ajjala:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **S. Pandey:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Abraham:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Kambhampati:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **T. Bandyala:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Bhatta:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **A. Shinde:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd.

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.06/N15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vivo* assessment of the locus coeruleus in young and older adults using neuromelanin-sensitive MRI

Authors: ***M. BETTS**[1], A. CARDENAS-BLANCO[1,4], M. KANOWSKI[5], K. FLIEßBACH[2,6], S. TEIPEL[3,7], F. JESSEN[2,8], E. DÜZEL[1,4];
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Psychosomatic Med., Rostock, Germany; [8]Univ. of Cologne, Dept. of Psychiatry, Cologne, Germany

Abstract: It has been proposed that the earliest pathology associated with Alzheimer's disease is the occurrence of abnormal tau in the locus coeruleus (LC) (Braak et al., 2011). Furthermore, there is clear evidence that LC tau pathology increases with age (Braak et al., 2011, 2015). Accompanying this effect is an age-related increase in neuromelanin, a pigmented polymer that results from the oxidation of noradrenaline and accumulates in the LC (Zucca et al., 2006). Thus MR sequences optimized to detect neuromelanin can be used to reliably locate the LC (Sasaki et al., 2006; Keren et al., 2009; Clewett et al., 2016) and may serve as a marker to assess structural changes in aging and disease. The aim of the work presented was to assess the signal intensity of the LC in young and healthy older adults using neuromelanin-sensitive MRI. We hypothesised older adults would demonstrate an increase in LC intensity owing to an age-related increase in neuromelanin. 25 young (22-30 years old; M:F 13:12) and 57 healthy elderly adults (61-80 years old; M:F 19:38) were scanned at 3T using a T1-weighted FLASH sequence (0.75mm isotropic resolution whole brain acquisition). In order to gain superior contrast and delineation of the LC, a study-wise template (Avants et al., 2008) was created from all subjects with good LC visualization (n=67). The LC and two unilateral reference regions in the left and right pons were subsequently segmented on the template and applied to all subjects. The remaining subjects with poor LC visualization were co-registered to the study-wise template and LC and reference masks were warped to each individual space for data extraction. LC signal intensity was determined as $LC_{CNR} = (LC_{intensity} - Pons_{intensity})/Pons_{intensity}$. A significant age-related increase was observed in right ($t_{80} = -2.21$, $p = <0.05$) and bilateral LC signal intensity ($t_{80} = -2.21$, $p = <0.05$). No significant age-related differences in mean intensity were observed in the left or right pons reference regions. Assessment of maximum (max) intensity voxels across all subjects revealed a significant unilateral difference, whereby greater signal intensity was observed in the left LC ($t_{81} = 4.73$, $p = <0.0001$). Significant age-related differences in LC max signal intensity were observed in left ($t_{81} = -2.73$, $p = <0.01$) and right ($t_{81} = -3.99$, $p = <0.0005$) LC hemispheres. No sex differences in mean or max LC signal intensity were observed. Our findings suggest estimating neuromelanin signal intensity may be a promising surrogate marker to assess the structural integrity of the human LC *in vivo*. Further studies are underway to assess how age-related accumulation of neuromelanin in the LC may impact memory and cognition.

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Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.07/N16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH (U01 AG043416)

Alzheimer's Association

Title: A translational program of AAV2-BDNF gene delivery into the entorhinal cortex for Alzheimer's disease: Development of MRI guidance for accurate gene targeting and distribution in non-human primates

Authors: *A. H. NAGAHARA[1], B. R. WILSON[1], I. IVASYK[1], I. KOVACS[1], S. RAWALJI[1], J. R. BRINGAS[2], P. J. PIVIROTTO[2], W. SAN SEBASTIAN[2], L. SAMARANCH[2], K. S. BANKIEWICZ[2], M. H. TUSZYNSKI[1,3];
[1]Neurosci, UC San Diego, La Jolla, CA; [2]Neurolog. Sci., UC San Francisco, San Francisco, CA; [3]Veterans Affairs Med. Ctr., San Diego, CA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease with early pathology occurring in the entorhinal cortex (EC). Our previous findings demonstrate that gene delivery using AAV2-Brain-Derived Neurotrophic Factor (BDNF) represents a potential neuroprotective therapy to prevent cortical neuronal loss in early AD (*Nat Med* 2009; *J Neurosci* 2013), and we are performing long-term safety studies in non-human primates prior to proposing human clinical trials. Accurate targeting of the entorhinal cortex is technically challenging due to its deep location on the ventral surface of the brain, together with atrophy of the EC in AD. To ensure accurate targeting and vector distribution in the EC, we used an MRI-guidance method with co-infusion of the MR contrast agent gadoteridol as a new method for gene delivery in AD. 8 rhesus macaques received AAV2-BDNF ($3x10^8$ vp/µl) combined with 1 mM gadoteridol (MRI tracer) using an MR-compatible cannula inserted through a ball-joint array port surgically implanted on the skull. MRI scans performed with a 3 Tesla scanner provided T1, T2, MP-RAGE imaging to plan and visualize vector delivery. Compared to non-MR guidance methods, real-time MR guidance with co-infusion of gadoteridol enabled substantial improvements in vector targeting and distribution in the EC. The spread of gadoteridol in the EC on MR scanning corresponded closely with the distribution of BDNF immunolableing post-mortem. The volume of the AAV2-BDNF infused significantly correlated with the number of BDNF labeled cells and the volume of BDNF immunoreactivity in the EC. Furthermore, BDNF labeling revealed widespread increase of BDNF in the hippocampus through anterograde transport. These results indicate that MRI-guidance provides a method for accurate AAV2-BDNF delivery to the EC and for extending the distribution of BDNF into the hippocampus. We conclude that MRI guidance is

likely to enhance the safety and accuracy of gene delivery for Alzheimer's disease and other disorders.

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Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.08/N17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant K23 AG038357 (KAV)

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Title: Relative incidence of seizures and myoclonus in Alzheimer's disease, dementia with Lewy bodies, and frontotemporal dementia

Authors: *S. DARWISH, A. J. BEAGLE, K. G. RANASINGHE, E. KARAGEORGIOU, K. A. VOSSEL;
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Abstract: Background: Patients with dementia have increased susceptibility to seizures and myoclonus. Pathomechanisms for network hyperexcitability involving distinct disease-related proteins and brain regions have been proposed. The relative incidence of seizures and myoclonus in the three most common neurodegenerative dementias, Alzheimer's disease (AD), dementia

with Lewy bodies (DLB), and frontotemporal dementia (FTD) spectrum, is unknown.
Objective: Establish incidence rates of first unprovoked seizures and myoclonus in patients with AD, DLB, and FTD at our center.
Methods: Records of patients meeting diagnostic criteria for AD (n= 1,319), DLB (n= 178), and FTD (n=348) from an eight year interval (2006-2013) were reviewed for evidence of new-onset seizures and myoclonus. Subjects with childhood seizures, provoked seizures (e.g., cortical lesion) or provoked myoclonus were excluded. Cumulative probabilities of developing seizures and myoclonus were calculated and compared between diagnostic groups, and age-stratified incidence rate ratios (IRR) were calculated using rates of age-matched control populations from epidemiological studies.
Results: The cumulative probability of developing seizures after disease onset was 13.4% in AD, 14.7% in DLB, and 3.0% in FTD ($p = 0.006$, age-stratified log-rank). The IRR was 9.7 for AD, 9.7 for DLB, and 6.3 for FTD. The cumulative probability of developing myoclonus was 44.2% in AD, 58.1% in DLB, and 24.7% in FTD ($p < 0.001$). Seizures increased with earlier age-at-onset in AD (age 30-49 IRR 86.6; 50-69 IRR 20.7; 70+ IRR 1.9; $p < 0.0001$) and myoclonus increased with earlier age-at-onset for patients in all three groups (AD $p < 0.001$; DLB $p = 0.02$; FTD $p = 0.003$). Seizures and myoclonus were significantly associated; 33.3% of patients with seizures had myoclonus, whereas 10.2% of patients without seizures had myoclonus ($p < 0.001$). Seizures began an average of 3.9 ± 4.0 years after the onset of cognitive decline, and myoclonus began 5.4 ± 3.1 years after onset.
Conclusions: Seizures and myoclonus occur with greater incidence in patients with AD, DLB, and FTD than in the general population, but the rate varies with diagnosis and age-at-onset, suggesting varied pathomechanisms for neural network hyperexcitability. Both symptoms tend to occur early in disease progression, indicating they could herald the onset of neurodegenerative disease. As myoclonus and seizures are treatable features of dementia, patient quality of life could be improved with their early recognition and treatment.

Disclosures: S. Darwish: None. **A.J. Beagle:** None. **K.G. Ranasinghe:** None. **E. Karageorgiou:** None. **K.A. Vossel:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.09/N18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG042890

Title: Localization of Peroxisome Proliferator Activated Receptor alpha in frontal cortex of Alzheimer's disease patients

Authors: *A. FRACASSI[1,2], S. MORENO[2], G. TAGLIALATELA[1];
[1]Mitchell Ctr. for Neurodegenerative Diseases, Dept. of Neurol., Univ. of Texas Med. Br., Galveston, TX; [2]Dept. of Sci., Univ. Roma Tre, Rome, Italy

Abstract: Alzheimer's disease (AD) is the most common form of dementia, particularly affecting the hippocampus and frontal cortex. The ensuing cognitive and memory impairments are associated with amyloid β-peptide (Aβ) accumulation, oxidative stress, altered lipid metabolism and inflammation. These mechanisms are reported to be regulated by a subfamily of nuclear receptors, referred to as Peroxisome Proliferator-Activated Receptors (PPARs), which include three isoforms α, β/δ and ɣ. Among these, PPARα has been suggested to play a neuroprotective role in acute and chronic brain pathologies. Indeed, *in vivo* and *in vitro* studies have demonstrated the beneficial effects of PPARα agonists in memory consolidation in AD models. In order to begin translating these observations in the actual AD brain, here we used immunofluorescence microscopy to study PPARα localization in the frontal cortex of AD patients (n=4) compared to control subjects (n=4). Our results showed a significant increase of PPARα in AD as compared to control. The enhanced expression of PPARα might represent a response to counteract the redox imbalance and consequent oxidative stress occurring during AD pathology. Indeed the expression of several antioxidant enzymes regulated by PPARα namely superoxide dismutases 1 and 2, catalase and glutathione peroxidase appears modulated. Interestingly both nuclear and cytosolic localization of PPARα was observed. Cytosolic localization is likely associated with non-genomic actions of PPARα known to reduce inflammatory responses. Double staining demonstrated a prevalent colocalization of PPARα with the astroglial marker GFAP as compared to the neuron-specific marker NeuN. This is observed in both control and AD brains, however with a significant increase in AD patients. Notably, PPARs activation has been reported to suppress the expression of proinflammatory genes, and considering the key role of astroglia in neuroinflammation our results suggest a compensatory action of PPARα in controlling inflammatory pathway in response to Aβ accumulation. On the basis of this preliminary study it appears that effective therapeutic intervention against neurodegeneration/neuroinflammation targeting PPARα and peroxisomes should be considered.

Disclosures: A. Fracassi: None. **S. Moreno:** None. **G. Taglialatela:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

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Program#/Poster#: 410.10/O1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Predicting co-variation of alzheimer's disease pathology from brain connectivity

Authors: *L. DIGMA[1], K. ARNEMANN[1], A. RAJ[2], W. JAGUST[1];
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Abstract: The accumulation of amyloid-β is one of the pathological features associated with Alzheimer's disease (AD). The mechanism by which amyloid-β spreads throughout the brain, however, is not well understood. One hypothesis is that the toxic agent may propagate to distributed areas across the brain via neural connections. In this study, we test this transneuronal spread hypothesis by comparing connectivity measures in young, healthy subjects to the distribution of amyloid-β across cognitively normal, older subjects that harbor a significant amount of pathology.

Resting-state fMRI data from healthy, young subjects (N=78, age=23.9±2.8) were used to measure functional connectivity using Pearson's correlations between 78 regions of interest from the Desikan-Killiany atlas. Diffusion weighted MRI data from an independent subset of healthy, young subjects (N=14, age=23.1±4.8) were used to estimate structural connectivity between these regions. PiB-PET imaging was used to measure amyloid-β deposition in a subset of cognitively normal, older subjects (N=29, age=76.9±4.8). We then calculated co-variation of amyloid-β using Pearson's correlations of PiB-DVRs between every pair of 78 regions. For each region, we performed linear regression to assess the relationship between its functional connectivity to other regions and amyloid-β co-variation between it and other regions. A bootstrapping procedure was then conducted to compare amyloid-β co-variation between structurally and non-structurally connected pairs of regions.

We found functional connectivity to be a strikingly good predictor of amyloid-β co-variation for 68 of 78 of regions (R^2=0.30±0.12, $p<0.05$, Bonferroni corrected), such that more strongly functionally connected regions exhibited higher amyloid-β co-variation. Moreover, we found amyloid-β co-variation to be significantly higher between structurally connected regions ($p<1e-4$).

In conclusion, the strong relationship between connectivity measures derived from young and amyloid-β co-variation across older subjects lends support to the notion that amyloid-β spreads through neural connections. Additional work, such as longitudinal assessment, is necessary to further investigate the spreading mechanisms of amyloid-β.

Disclosures: **L. Digma:** None. **K. Arnemann:** None. **A. Raj:** None. **W. Jagust:** None.

Poster

410. Alzheimer's Disease: Treatment in Humans

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Program#/Poster#: 410.11/O2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Supported by GliaCure, Inc

The Alzheimer's Drug Discovery Foundation

Title: A P2Y6 receptor pro-drug modulates cerebrospinal fluid amyloid β1-42 in PS1/APP mice and in patients with mild Alzheimer's disease

Authors: *J. DONG[1,2], R. DOYLE[1], R. SCHREIBER[3], N. ROULEAUX[1,4], K. FLICK[1], P. HAYDON[1,2];
[1]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [2]GliaCure, Inc., Boston, MA; [3]Suadeo Drug Discovery Consulting LLC, Charlestown, MA; [4]Psychology and Neuroscience, Sch. for Mental Hlth. and Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: The purinergic P2Y6 receptor stimulates microglial phagocytosis and has been identified as a potential molecular target for the treatment of Alzheimer's disease (AD). We have developed pro-drugs that yield agonists for this receptor and have evaluated their potential as a disease modifying treatment for AD. Aβ1-42 accumulates in the parenchyma and decreases in the cerebrospinal fluid (CSF) of both AD mouse models and patients. Our lead candidate, GC021109, was evaluated in a PS1/APP mouse model of AD. Preclinical studies revealed that GC021109 reduced plaque burden in the hippocampus and cortex, and simultaneously elevated Aβ1-42 in the CSF as well as restored memory formation. Investigational New Drug-enabling studies as well as a phase 1a single ascending dose study demonstrated that GC021109 was safe and well tolerated and exhibited dose proportional pharmacokinetics. In a double blind, placebo-controlled, phase 1b study, patients with mild to moderate AD (n=36) were dosed once daily with 1, 10 or 30mg of GC021109 for 28 days. CSF biomarkers of patients were analyzed at baseline and after 28 days of treatment. Similar to our preclinical studies, we observed a significant increase in Aβ1-42 in CSF following treatment. Patients with mild AD exhibited robust responses to GC021109 ($P<0.005$), while those with moderate AD were less responsive. Together these results suggest that GC021109 has the potential for a disease modifying treatment for AD.

Disclosures: **J. Dong:** 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 study is supported by GliaCure, Inc. and The Alzheimer's Drug Discovery Foundation. **R. Doyle:** None.

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Poster

410. Alzheimer's Disease: Treatment in Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 410.12/O3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The use of human-based methods to undertake new strategies for Alzheimer's disease research and define "pathways of disease"

Authors: *A. LAM[1,2], F. PISTOLLATO[3], E. L. OHAYON[1];
[1]Green Neurosci. Lab., Neurolinx Res. Inst., San Diego, CA; [2]Res. Policy, Physicians Committee for Responsible Med., Washington, DC; [3]Inst. for Hlth. and Consumer Protection (IHCP), European Commission – DG Joint Res. Ctr. (JRC), Ispra, Italy

Abstract: Previously we investigated the translational challenges in Alzheimer's disease (AD) due to reliance on reductive animal models and their failure to manifest the pathogenesis of AD and related dementias. Although there has been some recognition of this bias, the advancement and expansion of under-privileged human-based research directions will require some fundamental paradigm shifts and a global effort. Here we present key examples in human-based tissue studies, computational modeling, induced pluripotent stem research and multi-scale non-invasive readouts that can provide the foundational knowledge needed to define "pathways of disease" at the level of complexity necessary to bridge the clinical translation gap in AD. We will discuss pilot initiatives to develop resources to support human-based research, including proposals for novel human dementia tissue resources, an open online xeno-free induced pluripotent stem cell toolkit, and the adoption of a conceptual framework based on "Adverse Outcome Pathways" (AOP) as already envisioned in the field of toxicology and regulatory testing. We also describe how this multi-pronged approach will provide more predictive, adaptive, ethical and personalized precision care in response to the pathogenesis of AD and related dementias.

Disclosures: A. Lam: None. **F. Pistollato:** None. **E.L. Ohayon:** None.

Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

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Program#/Poster#: 411.01/O4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mechanisms of Aging and Dementia NIA Training Grant T32 AG20506

Title: β- and α-secretase processing of amyloid precursor protein in the human central nervous system

Authors: *J. A. DOBROWOLSKA ZAKARIA[1], R. J. BATEMAN[2], R. J. VASSAR[1];
[1]Cell & Mol. Biol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Washington Univ. in St. Louis, St. Louis, MO

Abstract: The amyloid hypothesis proposes that increased production or decreased clearance of amyloid-beta (Aβ) leads to higher order amyloid structures, such as oligomers and plaques that initiate a cascade of events, culminating in neuronal death which manifests as Alzheimer's disease (AD). Aβ is generated from the sequential cleavage of Amyloid Precursor Protein (APP) by β- and γ-secretase. APP, a transmembrane protein, may be processed in one of at least two pathways and is initially cleaved by either α-secretase or β-secretase (BACE1). α-secretase cleavage of APP, occurring more frequently under physiological conditions, precludes the formation of Aβ and produces non-toxic soluble APP-α (sAPPα). Alternatively, APP is initially cleaved by BACE1 releasing soluble APP-β (sAPPβ), and is subsequently cleaved by γ-secretase producing Aβ. Some studies found BACE1 protein and sAPPβ are increased in cerebrospinal fluid (CSF) and post-mortem AD brain. Our previous data demonstrate an increase in CSF sAPPβ: sAPPα ratio in AD subjects versus cognitively normal age-matched controls, indicating a shift toward BACE1 processing of APP under pathophysiological conditions. Further, a high positive correlation exists between sAPPβ and Aβ concentrations in human CSF. Additionally, a stable isotope labeling kinetics (SILK) study suggests that about 50% of AD patients may overproduce Aβ. Together these findings suggest increased BACE1 activity may cause increased Aβ in at least a subpopulation of AD patients, but this hasn't been assessed directly.
Using our previously developed highly sensitive SILK/immunoprecipitation/liquid chromatography-mass spectrometry methods, we are quantifying sAPPβ and sAPPα kinetics in CSF from human AD subjects and cognitively normal age-matched controls, in order to determine β- and α-secretase activity in human CNS. We hypothesize that approximately half of AD patients overproduce Aβ due to increased BACE1 activity, while the other half have decreased Aβ clearance. By directly measuring production rates of sAPPβ and sAPPα *in vivo*, we will determine if, and by how much, BACE1 activity is increased in AD subjects; this would allow for characterization of AD subpopulations most likely to benefit from BACE1 inhibitor

(BSI) treatment. These results will not only elucidate human CNS APP physiology and AD pathophysiology, but outcomes of this study may also prove useful for measuring pharmacodynamic effects of candidate therapeutics, such as BSIs. BACE1 is currently a high priority target for AD, and results of altered BACE1 activity in AD are critical for understanding AD pathophysiology and the development of disease modifying therapeutics.

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Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Kanae Foundation for the Promotion of Medical Science (MM)

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Title: *In vivo* spectral FRET assay for monitoring PS1/γ-secretase conformational changes

Authors: *M. MAESAKO, J. HORLACHER, O. BEREZOVSKA;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Using Förster resonance energy transfer (FRET)-based imaging techniques, we have previously shown that familial Alzheimer's disease (AD) mutations in Presenilin 1 (PS1) increase proximity of the PS1 N-terminus (NT) to the large cytoplasmic loop domain, causing so called "closed" pathogenic PS1 conformation. On the other hand, several nonsteroidal anti-inflammatory drugs and γ-secretase modulators, which are known to decrease the Aβ42/40 ratio, induce PS1 "open" conformation. This indicates that PS1 conformational changes are tightly linked to changes of the Aβ42/40 ratio, presenting pathogenic "closed" PS1 conformation as a potential target for AD prevention.
It is well known that Ca^{2+} overload is observed in AD mouse models, and we have recently reported that an increase in intracellular Ca^{2+} levels triggers PS1 pathogenic "closed" PS1 conformation in cultured neurons. However, whether Ca^{2+}-driven PS1 conformational changes similarly occur *in vivo* is unknown.
For monitoring PS1 conformational change, we have previously developed a conformation sensitive FRET probe: G-PS1-R, in which wild type PS1 with enhanced green fluorescent protein (GFP) fused to the NT and red fluorescent protein (RFP) inserted into the cytoplasmic loop.
Here we establish a new *in vivo* assay to monitor PS1 conformational change in living mouse brain. For this, we constructed a vector encoding G-PS1-R under human Synapsin1 promoter for targeted neuronal expression, and developed Adeno associate virus (AAV) to express G-PS1-R in mouse cortical neurons. To evoke higher Ca^{2+} levels in neurons, we topically applied KCl under cranial window. Using multi photon microscopy, we verified that G-PS1-R probe is sensitive to reliably reflect allosteric conformational change of PS1 *in vivo*.

Disclosures: **M. Maesako:** None. **J. Horlacher:** None. **O. Berezovska:** None.

Poster

411. Alzheimer's Disease: Secretases

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Japan Society for the Promotion of Science fellowship (MM)

Kyoto University Foundation fellowship (MM)

German National Academic Foundation fellowship (JH)

Title: Activity-driven PS1 phosphorylation is responsible for pathogenic conformational change of the PS1/gamma-secretase

Authors: *J. HORLACHER, M. MAESAKO, O. BEREZOVSKA;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Alzheimer's disease (AD) is a progressive, neurodegenerative brain disorder and together with other forms of dementia, it is one of the major causes of disability in later life. Senile plaques containing β-amyloid (Aβ) are a major pathological hallmark of AD. One of the enzymes mainly involved in the production of Aβ is Presenilin-1 (PS1)/gamma-secretase. More than 180 familial AD (fAD) mutations have been identified in its gene. Mostly all of them result in a similar biochemical, histological, and clinical phenotype – an increase in the Aβ42/40 ratio and early age of onset. As a mechanism of elevation of the Aβ42/40 ratio, we have shown that fAD mutant PS1 isoforms reveal pathogenic "closed" conformation. Such PS1 pathogenic conformational change was also observed in aging and in sAD brains, as determined by using Förster resonance energy transfer (FRET)-based imaging techniques.
It has been suggested that elevation of neuronal Ca^{2+} levels can enhance the production of Aβ, and Aβ42 in particular. But the underlying mechanisms remain unclear. We have recently reported that an increase in intracellular Ca^{2+} levels leads to a conformational change in PS1. This "closed" PS1 conformation, reflected by an increased proximity between N- and C-termini, is followed by up-regulation in the Aβ42/40 ratio. Here we investigate the mechanistic link between Ca^{2+} overload and PS1 pathogenic conformational change. First, we found that Ca^{2+} influx increases the level of phosphorylated PS1. Using phosphorylation-inhibited PS1 constructs in which serine (S)/ threonine (T), previously reported as a phosphorylation site, is substituted for alanine, we found that phosphorylation at three different domains in PS1 is involved in Ca^{2+} -driven PS1 pathogenic conformational shift. Furthermore, we quantified the amount of phosphorylated PS1 in human brains, and found that PS1 phosphorylation is significantly enhanced in the AD brains as compared to non-demented control brains. Collectively, our data indicate that Ca^{2+}-induced PS1 phosphorylation at the three domains may present a molecular mechanism of pathological conformational change in PS1/gamma-secretase, leading to amyloidogenesis.

Disclosures: J. Horlacher: None. **M. Maesako:** None. **O. Berezovska:** None.

Poster

411. Alzheimer's Disease: Secretases

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01 AG042513

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NIH/NIA P50 AG568132

Knight Alzheimer's Disease Research Center

Title: Phosphatase activity during sleep/wake cycles regulates APP processing and brain ISF amyloid-beta levels

Authors: C. E. WALLACE, H. M. EDWARDS, *J. R. CIRRITO;
Neurol., Washington Univ., Saint Louis, MO

Abstract: Alzheimer’s disease (AD) is initiated by the progressive accumulation of amyloid-beta (Abeta) peptide in the brain as toxic structures, such as Abeta oligomers and plaques. Evidence in both APP transgenic animal models and in humans suggests that brain Abeta levels fluctuate with the diurnal cycle; Abeta within the brain extracellular fluids are high during wakefulness and low during sleep. The diurnal fluctuation has been detected in the brain interstitial fluid (ISF) of APP transgenic mice and in the cerebrospinal fluid (CSF) of humans. In mice, increasing sleep pharmacologically suppresses brain ISF Abeta levels and, conversely, acute sleep deprivation increases ISF Abeta levels. Chronic alterations in sleep have a similar impact on amyloid plaque accumulation in these mice. We hypothesized that sleep is altering an intracellular signaling pathway that ultimately regulates Abeta generation and secretion into the brain ISF. We used in vivo microdialysis to measure brain ISF Abeta levels every hour over several days in living mice. Similar to previous studies, we detected a diurnal fluctuation in ISF Abeta levels during the sleep/wake cycle. Inhibiting Extracellular Regulated Kinase (ERK), increased ISF Abeta levels by 50% and blocked the fluctuation in ISF Abeta, suggesting that ERK plays a role in the diurnal rhythm of Abeta. Interestingly, ERK has been shown to be downstream of several neurotransmitter receptors (e.g. serotonin and NMDA receptors) which also regulate Abeta levels. SHP-2 is a phosphatase that dephosphorylates phospho-ERK to deactivate it. Inhibition of SHP-2 reduces ISF Abeta levels and also blocks the diurnal fluctuation. Our data suggest that there is an inverse relationship between the amount of phospho-ERK and the activity of SHP-2 which determines how much Abeta is produced in response to the physiological fluctuation in sleep/wake.

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Poster

411. Alzheimer's Disease: Secretases

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Research Funding for Longevity Sciences (25-20) from the National Center for Geriatrics and Gerontology (NCGG)

Title: Retromer and Rab2-dependent trafficking are involved in PS1 degradation by proteasomes in endocytic disturbance

Authors: ***N. KIMURA**[1], N. UEDA[1], T. TOMITA[2], K. YANAGISAWA[1];
[1]Dept. of Alzheimer's Dis. Res., Natl. Ctr. For Geriatrics and Gerontology, Aichi, Japan; [2]The Univ. of Tokyo, Tokyo, Japan

Abstract: Accumulating evidence shows that endocytic disturbance affects β-amyloid peptide (Aβ) cleavage from β-amyloid precursor protein (APP), suggesting that endocytic pathway deficits are involved in Alzheimer's disease pathogenesis. Presenilin-1 (PS1) is the catalytic core of the γ-secretase complex required for Aβ generation, and mutations of PS1 are the predominant cause of familial AD (FAD). Interestingly, several recent studies showed that PS1 mutations cause lysosomal abnormalities, suggesting that they may also cause endocytic disturbances. Most AD patients, however, do not have PS1 mutations. Thus, it remains unclear whether endocytic disturbances alone can affect PS1 localization and function. Previously, we showed that aging induces endocytic disturbance, resulting in the accumulation of Aβ and APP in enlarged endosomes. It remains unclear, however, whether PS1 localization and function are affected with endocytic disturbance. Thus we investigated the relationship between endocytic disturbance and PS1. In Neuro2a cells, we found that PS1 is transported from endosomes to the ER/Golgi compartments via retromer trafficking during endocytic disturbance. We also confirmed that PS1 interacts with vacuolar protein sorting-associated protein (VPS) 35 both *in vitro* and *in vivo*. Moreover, we found that PS1 is degraded by proteasomes via a Rab2-dependent trafficking pathway, only when endocytic disturbance occurs. These findings suggest that PS1 levels and localization within endosomes may be regulated by retromer trafficking and ER-associated degradation (ERAD), even if endocytic disturbance significantly induces the endosomal accumulation of APP and BACE1. Results of this study also suggest that retromer deficiency can affect PS1 localization in endosomes, where Aβ cleavage mainly occurs, possibly leading to enhanced Aβ pathology.

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Poster

411. Alzheimer's Disease: Secretases

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Title: Pen-2 plays a critical role in substrate binding

Authors: ***C. HU**[1], T. LI[2], J. XU[3], M. CUI[1], X. XU[1];
[1]Comparative and Exptl. Med., The Univ. of Tennessee, Knoxville, TN; [2]Tianjin Med. Univ., Tianjin, China; [3]Jilin Med. Univ., Jilin, China

Abstract: Presenilin enhancer-2 (Pen-2), presenilin-1 (PS1) or presenilin-2 (PS2), nicastrin (NCT), and anterior pharynx-defective 1 (Aph-1) have been considered the minimal essential components required to form an active γ-secretase complex. Specifically, PS is essential for substrates processing as the catalytic subunit of the complex. NCT has been reported to function as a substrate receptor. Aph-1 was believed to be essential to both assembly and maturation of the γ-secretase complex. Pen-2 was recognized as an important component for PS endoproteolysis to generate the N-terminal and C-terminal fragments of presenilin (PSN and PSC), which is a critical step in γ-secretase complex maturation. However, recent studies have shown that Pen-2 might be dispensable for PS endoproteolysis. Moreover, our recent study revealed that overexpression of both PS1N and PS1C failed to restore γ-secretase activity in Pen-2-knockout cells, indicating that Pen-2 might not be required only for PS endoproteolysis. Furthermore, our data demonstrated that deletion of Pen-2 had no effect on stabilization and dimer formation between PS1N and PS1C. These findings suggest that, in addition to PS

endoproteolysis of presenilin, Pen-2 may play a direct role in γ-secretase activity. To this end, our co-immunoprecipitation experiments strongly suggest that Pen-2 is essential for γ-secretase to interact with its substrates.

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Poster

411. Alzheimer's Disease: Secretases

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MEXT 262930110

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Title: Mechanism that BACE1 alternates the cleaving sites of human APP

Authors: ***T. SUZUKI**, A. KIMURA, S. HATA;
Hokkaido Univ., Sapporo-Shi, Japan

Abstract: APP is primarily cleaved at β-site (Asp1 of Aβ) by BACE1 to generate CTFβ/C99 from which Amyloid-β (Aβ) is produced. However, BACE1 alternatively cleave APP at β'-site (Glu11 of Aβ) to generate CTFβ'/C89, which is the amyloid lytic cleavage. It is important to understand a mechanism how BACE1 selects β- or β'-cleaving sites, because Aβ generation is the primary cause of Alzheimer's disease. We analyzed how BACE1 determines the cleavage site of APP. **Methods:** We first expressed mouse APP, and human APP with or without amino acid substitutions in N2a cells, and analyzed the generation of Aβ species (Aβ1-X and Aβ11-X) secreted into medium with immunoprecipitation-TOF/MS. We further analyzed localization of APP-CTFα and -CTFβ, C-terminal fragments of APP truncated by α-secretase (ADAM10/17) and BACE1 in mouse brain. We prepared the detergent (1% TritonX-100)-resistant membrane (DRM) and non-DRM fractions of mice brain and analyzed the membrane micro-localization of CTFα, CTFβ and CTFβ' by Western blotting. **Results:** N2a cells expressing mouse APP secreted lower levels of Aβ1-40 along with higher levels of Aβ11-40. By contrast, cells expressing human APP secreted higher levels of Aβ1-40 and lower levels of Aβ11-40. These results indicate that a major cleavage site of mouse APP is Glu11 site and that of human APP is Asp1 site. Two amino acids located on Aβ sequence are important to select Asp1 cleavage site. DRM fractionation study with mice brain shows that APP-CTFβ localized differently from APP-CTFβ' in

membrane fraction, suggesting that APP is cleaved by BASE1 in various membrane micro-regions. **Conclusions:** Major cleavage site of human APP by BACE1 is different from it of mouse APP, and f this alternative cleavage is caused by a difference of amino acid at two positions of Aβ sequence, contributing for selection of either β-site or β'-site. Studies with DRM fraction suggest that the alternative cleavage of human APP by BACE1 is also due to a cellular and membrane microenvironment in which APP and BACE1 localize.

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Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 411.08/O11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: BACE1/BACE2 selectivity, the next frontier in beta-secretase inhibition for Alzheimer's disease: A mouse model of depilation-induced pigmentation for *In vivo* screening

Authors: *P. H. WEN[1], J. BRADLEY[1], M. JOHNSON[2], M. SOTO[3], M. BOURBEAU[4], D. HICKMAN[3], S. WOOD[1];
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Abstract: BACE1 is the most promising drug target for Alzheimer's disease as it mediates the initial cleavage of the amyloid precursor protein in the generation of pathogenic Abeta peptides. Numerous BACE1 inhibitors possessing satisfactory drug-like properties have been discovered, yet it has proven challenging to design potent molecules that are selective against other aspartyl proteases including the closely related homolog, BACE2. Recent investigations into the physiological functions of BACE2 have aided in this task. PMEL17 (Pigment cell-specific Melanocyte protein), a transmembrane protein involved in melanogenesis, has been identified as a novel BACE2 substrate. Proteolytic cleavage of PMEL17 by BACE2 releases fragments into melanosomes, mediating formation of a matrix of amyloid fibers required for melanin deposition. This may explain the hypopigmentation phenotype observed in BACE2 KO mice, in which defective PMEL17 processing could lead to abnormal maturation of melanosomes. Moreover, recent studies showed that chronic treatment of wild type mice with a non-selective BACE1 inhibitor causes hypopigmentation of the fur and skin, implicating BACE2 as a tissue-specific protease and an important pigmentation gene. In addition, BACE2+/- and BACE2-/- mice treated with a potent dual BACE1/BACE2 inhibitor display dose-dependent appearance of irreversibly hypopigmented hair. To identify best-in-class BACE1 inhibitors that are selective

over BACE2 we have developed novel in vitro and vivo screening assays. A high-throughput cell-based assay was established to monitor BACE2-mediated turnover of known BACE2 substrates. Pharmacodynamic effects of BACE1 inhibitors have been assessed in a mouse model of depilation-induced hair follicle cycling. Multiple genes required for melanogenesis, including tyrosinase, TRP1 & 2 and MITF, as well as protein levels of PMEL17 cleavage products were profiled in the skin at stages of the hair cycle following depilation in mice treated with BACE1 inhibitors.

Disclosures: P.H. Wen: None. **J. Bradley:** None. **M. Johnson:** None. **M. Soto:** None. **M. Bourbeau:** None. **D. Hickman:** None. **S. Wood:** None.

Poster

411. Alzheimer's Disease: Secretases

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Program#/Poster#: 411.09/O12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG0145264

Title: Palmitate-enriched diet increases BACE1 expression and ensuing Amyloid-Beta genesis by evoking ER stress and subsequent CHOP activation

Authors: *G. A. MARWARHA, J. SCHOMMER, J. LILEK, O. GHRIBI;
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Abstract: Alzheimer’s disease (AD) is the most common form of dementia in the elderly that is histo-pathologically characterized by extracellular accumulation of aggregated Amyloid-β (Aβ) peptide as *neuritic senile plaques* and the intracellular accumulation of aggregated hyperphosphorylated protein tau (τ) as *neurofibrillary tangles.* The aspartyl protease BACE1 is indispensable for the engenderment of Aβ and catalyzes the rate-limiting step in Aβ genesis from AβPP. The expression of BACE1 protein as well as its enzymatic activity is significantly augmented in the AD brain. The etiology of AD is multifactorial and egregiously comprehended, but epidemiological studies have implicated a diet rich in saturated free fatty acids (sFFA) as a significant risk factor for developing AD. Palmitic acid (palmitate) is the most abundant long-chain free saturated fatty acid in the brain and the diet and higher palmitate levels in the plasma, as observed in obesity and diabetes, are inversely correlate with cognitive function. Recent cogent evidence has implicated endoplasmic reticulum (ER) stress as one of the culpable factors in initiating and fostering the deleterious neurodegenerative changes in AD. A multitude of studies have cogently demonstrated that sFFA such as palmitic acid evoke ER stress. Sustained

ER stress culminates in the increased expression of the transcription factor C/EBP Homologous Protein (CHOP, also called GADD153 or DDIT3). In addition to transcriptionally up-regulating the expression of a plethora of genes involved in ER stress response, increased expression of CHOP also enhances NF-κB signaling and transcriptional activity. NF-κB signaling and transcriptional activity positively and directly regulate the transcription of BACE1 and our earlier studies have demonstrated and unveiled a novel CHOP - NF-κB signaling pathway in the regulation of BACE1 expression. However, the extent to which palmitic modulates these pathways to impinge on BACE1 expression and subsequent Aβ genesis is not known. In this study, we determined the impact of high palmitate-diet on BACE1 expression and Aβ genesis and delineated the molecular mechanisms involved.

Disclosures: **G.A. Marwarha:** None. **J. Schommer:** None. **J. Lilek:** None. **O. Ghribi:** None.

Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 411.10/O13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Merck Research Laboratories

Title: Loss of BACE1 - but not BACE2 - function in mice results in decreased body weight, protection against diet-induced obesity and reduced anxiety.

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Abstract: BACE1 (β-site amyloid precursor protein-cleaving enzyme 1) is a key enzyme in the production of toxic amyloid peptides and is highly expressed in the brain, but also to a lesser extent in major peripheral organs such as muscle and liver. In contrast, BACE2 is mainly expressed in peripheral tissues and enriched in pancreatic β cells where it regulates its cell function and mass. Previous reports demonstrated that loss of BACE1 function decreases body weight, protects against diet-induced obesity and enhances insulin sensitivity in mice (Meakin et al, Biochem. J. 441, 2875-296, 2012), whereas mice lacking Bace2 exhibit reduced blood glucose levels, improved intraperitoneal glucose tolerance and increased β cell mass (Esterhazy et al, Cell Metabol. 14, 365-377, 2011). Impaired glucose homeostasis and insulin resistance are

hallmarks of Type 2 diabetes, which is an important risk factor for AD (Alzheimer's disease). Therefore, we tested the contribution of the individual BACE isoforms to those metabolic phenotypes by placing Bace1 knockout (KO), Bace2 KO, Bace1/2 double knockout (dKO) and wild type (WT) mice on a high fat diet for 16 weeks. Bace 1 KO and Bace1/2 dKO mice showed decreased body weight, improved glucose tolerance and enhanced insulin resistance vs WT mice. Surprisingly, Bace2 KO mice did not show any significant differences in body weight, glucose tolerance or insulin resistance under our experimental conditions, indicating that lack of Bace1 - but not Bace2 - function contributes mainly to the metabolic phenotypic changes observed in Bace1/2 dKO mice. Although Bace1 KO, Bace2 KO and Bace1/2 dKO mice were normal in most behavioral assays, both the Bace1 KO and Bace1/2 dKO - but not the Bace2 KO - mice showed reduced anxiety. Only the Bace1/2 dKO mice were hyperactive in the open field and other locomotor activity assays during the light phase. Finally, chronic treatment of C57BL6 mice with 10 mg/kg/day of the dual BACE1/2 inhibitors MBi-3 or MBi-9 mixed in a HFD for 3 weeks resulted in a modest improvement of glucose tolerance. Our data suggest that inhibition of BACE1 has the greater role (vs BACE2) on any potential metabolic homeostasis improvements or anxiety behavior.

Disclosures: **T.W. Rosahl:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **L.A. Hyde:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **M. Champy:** None. **H. Meziane:** None. **C. Canasto-Chibuque1:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **K. Juhl:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **Z. Li:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **B. Petit-Demouliere:** None. **T. Sorg:** None. **J. Scott:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **G.J. Eiermann:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **J.N. Cumming:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **E.M. Parker:** A. Employment/Salary (full or part-time): Merck Research Laboratories. **M.E. Kennedy:** A. Employment/Salary (full or part-time): Merck Research Laboratories.

Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 411.11/O14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KU2016

Title: Role of PSEN1 in neuronal differentiation and Alzheimer's disease - modeling human neurodegeneration in a dish

Authors: *C. PIRES[1], A. POON[1], B. SCHMID[2], T. T. NIELSEN[3], L. E. HJERMIND[3], J. NIELSEN[3], C. CLAUSEN[2], P. HYTTEL[1], B. HOLST[2], K. FREUDE[1];
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Abstract: Alzheimer's disease (AD) is the most common type of dementia and targets the cerebral cortex and certain subcortical regions. The familial (FAD) form shows early-onset symptoms and it is attributed to mutations in presenilin (PSEN) 1 and 2 and amyloid precursor protein (APP) genes. PSENs are constituents of the gamma-secretase complex and it correlates with increased toxic Aβ42 and senile plaques in AD brain. Sporadic (SAD) type has a late-onset and no causative genetic background has been identified except for apolipoprotein E (APOE) risk factor.
Human induced Pluripotent Stem Cells (hiPSC) allow for the generation of patient-specific neurons, providing *in vitro* tools for studying neurodegeneration. We have generated patient-derived PSEN1-hiPSCs carrying one of five different PSEN1 mutations (A79V, L150P, M146I, H214R and L282F). These PSEN1-hiPSCs were carefully characterized in regards to pluripotency by quantitative PCR and immunocytochemistry for key pluripotency genes/proteins. Furthermore, their differentiation potential was explored by embryoid-body formation and differentiation into all three germlayers. To assess even subtle disease phenotypes we have generated isogenic controls for each line via genome editing, by using the CRISPR-Cas9 system combined with ssODNs carrying the corrected nucleotide and inserted silent mutations. We are currently assessing disease phenotypes caused by different PSEN1 mutations including apoptosis (Caspase 3/7), cell ROS and autophagy assays, ELISAs to determine the relative increase in Aβ42 over Aβ40, spontaneous action potential and mitochondrial dysfunction, which is related to the "Ca^{2+} hypothesis" in AD. Moreover, terminally differentiated neurons from PSEN1, isogenic and sex/age-matched control lines are being analyzed via RNAseq to evaluate possible biomarker candidates, which will then be assessed/validated in the respective patients' CSF and blood samples, and also to evaluate if different mutation sites lead to distinct pathway alterations. Proteomics and metabolomics will also be studied so that a broad and detailed idea of the mutations' impact is constructed.

Disclosures: C. Pires: None. **A. Poon:** None. **B. Schmid:** None. **T.T. Nielsen:** None. **L.E. Hjermind:** None. **J. Nielsen:** None. **C. Clausen:** None. **P. Hyttel:** None. **B. Holst:** None. **K. Freude:** None.

Poster

411. Alzheimer's Disease: Secretases

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG026660

Title: Hif1alpha activates gamma-secretase and increases production of amyloid-beta in cells

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Abstract: Alzheimer's disease (AD) is the sixth leading cause of death in the US, and there is currently no effective treatment or cure. One major risk factor for developing this disease is hypoxia, or low oxygen conditions, often times developed after the occurrence of a stroke. The brain is especially vulnerable to these changes in oxygen level, as it has the highest oxygen demand in the body. Understanding the relationship between hypoxia and AD could help guide future therapeutics. Amyloid precursor protein (APP) is first cleaved by either alpha or beta secretase, followed by cleavage by gamma-secretase. If APP is first processed by beta secretase, cleavage by gamma-secretase produces amyloid beta peptides of varying lengths. These peptides can then oligomerize and form the hallmark plaques found in AD brains.Previous reports have shown that hypoxia increases amyloid-beta production in both cells and mice. We aim to show that this increase in amyloid-beta is due to an increase in gamma-secretase activity. Hif1alpha, the master regulator of hypoxia, directly binds to gamma-secretase, increasing its activity and production of amyloid-beta, independent of its canonical, transcriptional role.

Disclosures: C. Carroll: None. **Y. Li:** None.

Poster

411. Alzheimer's Disease: Secretases

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AMED

MEXT

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T264617450

T254617680

T243902830

Title: A brain-derived Abeta42 surrogate marker in peripheral blood

Authors: *S. TAGAMI[1], K. YANAGIDA[1], T. TOMONAGA[2], T. IKEUCHI[3], M. WARAGAI[4], M. TAKEDA[5], M. IKEDA[1], M. OKOCHI[1];
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Abstract: Objective: Amyloid beta42 in cerebrospinal fluid (CSF) is well established biomarker in Alzheimer's disease(AD). The value is utilized for diagnosis of preclinical AD and also for an indicator of therapeutic effect. However, Abeta42 in peripheral blood is not practically used as generally as that in CSF. Thus, as for a blood-based biomarker of AD, surrogate markers are necessary by which the level of brain Abeta42 generation can be estimated. We discovered APL1beta28, an Abeta42 surrogate marker, in human CSF and showed the APL1beta28 ratio (APL1beta28/total APL1beta) was elevated in CSF samples of FAD with PS1 pathogenic mutations, sporadic AD and MCI patients. Thus, the APL1beta28 ratio will be a biomarker which indicates Abeta42 generation in brain. To use this surrogate marker in large clinical studies, we have tried to detect and measure the level of this CNS peptide in peripheral blood.
Methods: We performed several biochemical treatments to purify plasma APL1beta28 and measure the levels by using LC/MS/MS system.
Results: We could successfully measure the level of the CNS APL1beta28 peptide in plasma. The concentration of APL1beta28 is 0.5pM, which is much less than that in CSF (500pM).
Conclusions: We have tried to develop Abeta42 surrogate marker in peripheral blood. The plasma APL1beta;28 ratio may be used to estimate the Abeta42 ratio in brain.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 412.01/O17

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Impaired synaptic transmission in the CA1 area of hippocampal slices of APPSwDutIowa/Nos2$^{-/-}$ (CVN) mice

Authors: M. V. KOPANITSA, J. PUOLIVÄLI, O. KONTKANEN, *R. HODGSON, A. NURMI, P. J. SWEENEY;
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Abstract: The CVN mouse model of Alzheimer's disease combines overexpression of the APP isoform 770 transgene that bears Swedish (K670N/M671L), Dutch (E693Q), and Iowa (D694N) mutations with a constitutive deletion of nitric oxide synthase 2. In addition to amyloid deposition, these mice exhibit aggregation of hyperphosphorylated tau and substantial neuronal loss. One of the brain areas that is profoundly affected by AD-like pathologies in CVN mice is the hippocampus. However, electrophysiological evidence of potential repercussions of excessive amyloid deposition and expression of hyperphosphorylated tau for synaptic transmission in the hippocampus has been lacking so far. To this end, we prepared hippocampal slices from 17-18-month-old CVN and aged matched control mice and, by using multi-electrode arrays (MEAs), recorded field excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA1 area in response to stimulation of Schäffer collaterals. When recording input-output relationships, we noted that peak fEPSP amplitudes were smaller in slices from CVN mice, particularly at higher values of stimulus strength. The maximum fEPSPs comprised 3.3 ± 0.8 mV in slices from control animals, whereas in CVN mice responses were slightly but significantly lower (2.7 ± 0.7 mV; $P = 0.015$; 2-way nested ANOVA, main genotype effect). In addition, paired-pulse facilitation (PPF) at an interpulse interval of 50 ms was lower in slices from CVN mice (159.9 ± 2.2%) than in control mice (170.4 ± 4.3%, $P = 0.044$).Short theta-burst stimulation elicited stable long-term potentiation (LTP) of fEPSP amplitudes in slices from CVN and control animals. Sixty-five min after induction, LTP levels were similar in both genotypes (CVN: 151.0 ± 4.2%; control: 152.5 ± 3.3%; $P > 0.05$). We conclude that a moderate impairment of basal synaptic transmission parameters in hippocampal slices from CVN mice is probably a reflection of a neuronal loss in the hippocampus. Disturbances in PPF may be also partly linked to selective degradation of some populations of hippocampal interneurons previously observed in this model.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI Grant-in-Aid for Young Scientists (B) (23700429)

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KAKENHI Grant-in-Aid for Young Scientists (B) (16K21328)

Title: GSK-3β-mediated phosphorylation of PICK1 regulates the GluA2-PICK1 interaction.

Authors: *S. YAGISHITA[1,2], M. MURAYAMA[2], T. EBIHARA[1], K. MARUYAMA[1], A. TAKASHIMA[2,3];
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Abstract: Alzheimer disease (AD) is the most common type of dementia, and the mechanisms leading to the onset of AD has been unknown. The hallmarks of AD are the deposition of amyloid β-protein (Aβ) and hyperphosphorylated tau, both of which have been shown to be related to long-term depression (LTD): Aβ dimers induce LTD, and the presence of tau is required for LTD induction. On the other hand, activation of GSK-3β, a critical molecular link between Aβ and tau, is required for LTD induction. Thus LTD has been recently regarded as an AD-related synaptic event.
LTD involves the removal of AMPA receptor subunit GluA2 from the synaptic plasma membrane. For the removal, GluA2 is required to interact with Protein interacting with C kinase 1 (PICK1). Here, we aimed to elucidate the relationships between GluA2, PICK1, GSK-3β, and tau.
We showed that GSK-3β phosphorylated PICK1 and that this phosphorylation promoted the GluA2-PICK1 interaction. We identified GSK-3β-mediated phosphorylation sites of PICK1. In particular, replacing the most C-terminal Ser416 with Ala disrupted the GluA2-PICK1 interaction, while substituting Ser416 with Glu or Asp retained this interaction, suggesting that phosphorylation at Ser416 residue was essential for the GluA2-PICK1 interaction. We also found that the presence of tau enhanced the GluA2-PICK1 interaction. However, the deletion of Ser416 did not affect the GluA2-PICK1 interaction, and the substitution of Ser416 with Ala did not alter the PICK1-PICK1 interaction. Using image analysis in COS-7 cells with GFP-fused PICK1, we showed that substitution of Ser416 with Ala increased the formation of GFP-positive

clusters, suggesting an increase in the association of PICK1 with the membrane. This may have resulted in the dissociation of the GluA2-PICK1 complexes.
This study proposes a new model for regulating the GluA2-PICK1 interaction and provides new insights into the molecular mechanisms leading to the onset of AD.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Alzheimer's Association GrantNIRG-15-363477

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BrightFocus Foundation Grant A2015535S

Title: Early manifestation of synaptic failure in a transgenic model of AD

Authors: ***S. FORNER**[1], A. G. PRIETO[1], A. LIMON-RUIZ[1], D. KONG-LEE[1], A. C. MARTINI[1], C. DA CUNHA[1], L. TRUJILLO-ESTRADA[1], R. AGER[2], J. C. DAVILA[3], A. GUTIERREZ-PEREZ[3], C. W. COTMAN[2], D. BAGLIETTO-VARGAS[2], F. M. LAFERLA[2]; [1]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [3]Dept. de Biología Celular, Univ. de Málaga, Málaga, Spain

Abstract: Alzheimer’s disease (AD) is a devastating neurodegenerative disorder clinically characterized by gradual and progressive memory and cognitive decline. AD is currently conceptualized as a disease of synaptic failure, and novel clinical and animal model studies indicates that synaptic impairments are the most robust pathological feature that better correlates with dementia, implicating it as important to the disease process. However, the underlying molecular mechanisms that impair the synaptic function and cause synaptic loss in AD is poorly understood, especially at early stage of the disease. Hence, studies designed to elucidate the mechanisms by which the synaptic function is affected early in AD will provide greater insight into the onset of cognitive deficits in AD patients, and it will allow identifying novel treatment targets. Here, we study in a preclinical AD model, the 3xTg-AD, how the synapses are

compromised early in the disease. Our study reveals that 3xTg-AD display significant reduction in the spine densities and important structural change at 6-7 month of age. These alterations are associated with Long-Term Potentiation (LTP) deficits and alterations in synaptic receptor trafficking. These important synaptic alterations are associated with elevated level of Aβ and tau pathology in the synapses. Finally, the levels of actin-related proteins are diminished in 3xTg-AD mice, which are crucial to maintain the shape and function of the synapse. Overall, our study indicates that synaptic alterations occur early in the pathobiology of AD and elucidating these mechanisms might offer novel therapeutic targets for AD patients.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Program#/Poster#: 412.04/P2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Early changes in structure and function of gray matter axons in a mouse model of Alzheimer’s disease

Authors: D. PEKALA, *M. RAASTAD;
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Abstract: Gray matter axons (GMAs) are the most common type of axon in the mammalian cortex. These axons are unmyelinated, are extremely thin (diameter on average 0.17 μm), and form *en passant* synapses at boutons distributed along their entire length. The function of GMAs involves action potential propagation and transmitter release. The specific morphology together with complex function indicates vulnerability of GMAs to pathological conditions. We have developed a method which allows to investigate structural changes of GMAs during the process of neurodegeneration in Alzheimer’s disease (AD). Together with changes in morphology we have also followed impact of AD on function of these axons. Experiments were performed on APP/PS1 transgenic mice that co-express five familial Alzheimer’s disease mutations (5xFAD) and WT controls. In order to detect structural changes, GMAs in hippocampus and cerebellum were labelled with lipophilic dye (DiI). Next, we measured intensity profiles of typical GMAs and estimated the ‘**bouton contrast**’ between each bouton and its adjacent thin axon and calculated the inter-bouton interval (**IBI**). To study functional changes, we investigated short-

term modulation of synaptic strength in two pathways of hippocampus: stratum radiatum (SR) and stratum lacunosum-moleculare (SLM). Extracellular recordings of field synaptic potentials (fEPSP) were performed on 300 μm transverse slices at 36 °C in the presence of APV (50 μM) in recording solution, to reduce the postsynaptic modulation of synaptic strength. Two months before symptoms or amyloid deposits have been reported in these mice bouton contrast was higher in 5xFAD compared to WT mice in axons from hippocampus and cerebellum. The IBI of hippocampal GMAs were longer in 5xFAD than WT controls. Boutons on hippocampal axons tended to be more regularly spaced in 5xFAD than in WT, while cerebellar axons were not different in the two groups. Recordings of fEPSP showed that synaptic facilitation was reduced in the 5xFAD in both SR and SLM compared to WT. These findings combined with the structural changes strongly support the hypothesis that GMAs change early during neurodegeneration.

Disclosures: D. Pekala: None. **M. Raastad:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS84324

"la Caixa" foundation fellowship to E. Vicario-Orri

Title: Genetic targeting of APP in hippocampus demonstrates that synapses postsynaptic to neurons expressing APP are the earliest sites of injury

Authors: *E. VICARIO ORRI, K. CHIANG, S.-H. TYAN, S. LEUTGEB, E. H. KOO; Neurosciences, Univ. of California San Diego, LA Jolla, CA

Abstract: The mechanisms by which Aβ causes synaptic dysfunction in Alzheimer's disease (AD) are not well understood. To date, it is not possible to determine whether Aβ-induced synaptic injury is initiated by the pre- or post-synaptic neurons and how injury is propagated. To address these questions, we targeted the amyloid precursor protein (APP) to CA1 or CA3 neurons by combining Cre/loxP and Tet-Off systems, thus providing temporal and spatial control of APP expression. Synapse loss was quantified by immunostaining of synaptic markers in dendritic layers of CA1 and CA3 neurons. In 12-month-old CA3-3xTg mice, where APP was expressed selectively in CA3 neurons, there was a significant 9% decrease in synaptophysin and

PSD-95 puncta only in the areas where axons from CA3 neurons synapse onto dendrites of CA1 neurons. This is consistent with our previous observation that LTP was impaired only in synapses where APP was expressed in pre- but not postsynaptic neurons. In 18-month-old animals, significant reductions in synaptophysin and PSD-95 (11-13%) were observed in dendritic fields of axons from CA1 as well as of CA3 neurons indicating a spread of injury also to synapses that were presynaptic to neurons expressing APP. Treatment with doxycycline for two weeks reversed both synaptic loss and LTP deficits in 12-month-old CA3-3xTg mice. Consistent with these observations, preliminary studies in 10-month-old CA1-3xTg mice did not reveal any synapse loss in CA1 dendritic fields but a trend towards a decrease in the subiculum. These results show that synapses most vulnerable to Aβ-induced injury are located postsynaptic to where APP was expressed. Interestingly, our data suggest that in this rodent model, synaptic injury with respect to reduction in LTP and synapse loss is not irreversible.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS 084324

Title: Aβ-induced caspase activation and synaptic injury from APP C99 fragment is prevented in the APP D664A mutant that inhibits caspase-mediated cleavage

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Abstract: Loss of synapses is a characteristic feature of Alzheimer’s disease (AD) and is believed to underlie the cognitive impairment in AD patients. The precise mechanisms of synaptic dysfunction and synapse loss are not well understood although it has been hypothesized that amyloid-β protein (Aβ) contributes to this process. We have previously proposed that an amyloid precursor protein (APP)-dependent pathway of Aβ toxicity related to cleavage of APP by caspases or caspase-like proteases at position 664 (APP695 numbering) contributes to synaptic injury and neuronal death. Here, we further examined the contribution of this pathway

to synaptic injury in organotypic neuronal slice cultures expressing APP D664A mutation designed to block cleavage of APP by caspases. Using a method previously reported to demonstrate Aβ-mediated synaptic depression in organotypic slice cultures, we confirmed a reduction in AMPA- and NMDA-mediated currents after expression of wt C99 fragment. However, this impairment was absent in neurons expressing C99 D664A, indicating that replacing the aspartate residue at position 664 to render APP non-cleavable is neuroprotective to Aβ-mediated neuronal toxicity. Interestingly, caspase 3 activity, as assayed by DEVD-afc cleavage, was elevated in slice cultures infected with wt C99, but was reduced in cultures infected with D664A mutant. This reduction was also seen in western blotting of activated caspase 3 and 6 as well as by the presence of caspase cleavage products (actin, synaptophysin and GADPH) in C99 wt but not D664A infected slice cultures. Lastly, incubation of purified caspases (3, 4, 6, 7, 8 and 9) with APP and C99 immunoprecipitated from HEK293 cells indicated that caspase 3 exhibited the highest activity in cleaving APP at position 664 in vitro. Our results demonstrated that neurons expressing C99 D664A were more resistant to synaptic injury than neurons expressing wt C99, consistent with the hypothesis that APP cleavage leads to the release of the putative C31 peptide from the APP C-terminus. Interestingly, the D664A mutant appears to block the activation of caspases detected after expression of C99, suggesting that the release of C31 peptide may be one mechanism by which Aβ is synaptotoxic. However, the latter cannot be ascertained from the current approach because the D664A substitution may have altered other pathways unrelated to C31 that contribute to neuronal injury.

Disclosures: **G. Park:** None. **B. Midthune:** None. **M. Navarro:** None. **R. Malinow:** None. **G. Salvesen:** None. **E.H. Koo:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 412.07/P5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Fingolimod modulates NMDA receptor properties in rat hippocampal slices

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Abstract: Sphingosine-1-phosphate (S1P) is a ceramide derivative serving not only as a regulator of immune properties but also as a modulator of synaptic plasticity in the brain. To better understand the mechanism underlying the effects of S1P on brain plasticity, we

investigated the potential impact of S1P receptor activation on NMDA receptor subunits. We used acute rat hippocampal slices as a model system, and determined the effects of the active phosphorylated S1P analog, fingolimod (or FTY720P). Treatment with FTY720P significantly increased phosphorylation of GluN2B-containing NMDA receptors at Tyr1472. This effect appears rather specific, as treatment with FTY720P did not modify other phosphorylation sites of GluN receptors. Pre-treatment of hippocampal slices with the compounds W146 and PP1 indicated that FTY720P-induced GluN2B phosphorylation at Tyr1472 epitopes was dependent on activation of S1P receptor subunit 1 (S1PR1) and Src/Fyn kinase, respectively. Cell surface biotinylation experiments indicated that FTY720P-induced GluN2B phosphorylation at Tyr1472 was also associated with increased levels of GluN1 and GluN2B subunits on membrane surface, whereas no change was observed for GluN2A subunits. These results suggest that activation of S1P receptors might enhance synaptic plasticity in the hippocampus by upregulating GluN2B phosphorylation in rat hippocampal slices, resulting in increased levels of receptors in neuronal membranes.

Disclosures: S. Attiori Essis: None. **M. Laurier-Laurin:** None. **É. Pépin:** None. **M. Cyr:** None. **G. Massicotte:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grants Council of Hong Kong SAR (HKUST 661013 and 16102715)

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Shenzhen Peacock Plan

S.H. Ho Foundation

Title: Melanocortin 4 receptor activation ameliorates synaptic plasticity impairment in a mouse model of Alzheimer’s disease

Authors: *M. TIAN[1,2,3], Y. SHEN[1,2,3], F. GONG[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3];
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Amyloid-beta (Aβ) accumulation leads to synaptic plasticity impairment and cognitive dysfunction in Alzheimer's disease (AD). We previously demonstrated that the G-protein-coupled receptor melanocortin 4 receptor (MC4R) is involved in the regulation of synaptic plasticity in the mouse hippocampus. Here, we show that the expression of the endogenous agonist of MC4R precursor polypeptide, pro-opiomelanocortin (POMC), changes along aging in the hippocampi of APP/PS1 mice, an AD mouse model. Interestingly, while the percentage of mature dendritic spines and spine size were reduced in the hippocampal cornu ammonis 1 (CA1) region in APP/PS1 mice, delivery of the MC4R agonist D-Tyr MTII reversed these defects. Furthermore, MC4R activation alleviated hippocampal synaptic plasticity impairment (i.e., long-term potentiation) in APP/PS1 mice. Interestingly, MC4R knockdown in the APP/PS1 mouse hippocampus abolished the rescue effect of D-Tyr MTII, suggesting that the specific activation of MC4R is critical for restoring synaptic functions in APP/PS1 mice. Our findings collectively demonstrate that activation of MC4R signaling in the hippocampus ameliorates the impaired synaptic plasticity in APP/PS1 mice, suggesting that MC4R signaling pathway might present potential targets for therapeutic interventions for AD.

Disclosures: **M. Tian:** None. **Y. Shen:** None. **F. Gong:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Support: Research Grants Council of Hong Kong SAR (HKUST 16102715)

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the Shenzhen Peacock Plan

the S.H. Ho Foundation

Title: MC4R activation alleviates amyloid-beta-induced synaptic dysfuntion

Authors: *F. GONG[1,2,3], Y. SHEN[1,2,3], M. TIAN[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3];
[1]Div. of Life Sci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China; [2]Mol. Neurosci. Ctr., [3]Sate Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong kong, China

Abstract: Activation of melanocortin 4 receptor (MC4R), which is well known for its role in regulating energy homeostasis, enhances synaptic plasticity in mouse hippocampus via the cAMP/PKA-dependent pathway. In Alzheimer's disease, impaired hippocampal synaptic plasticity is suggested to be induced by soluble amyloid-beta. Here, we show that treatment of cultured hippocampal neurons with amyloid-beta reduced synaptic transmission, and the reduction could be reversed by MC4R activation. Furthermore, the rescue action of MC4R at synapses was mediated via the cAMP/PKA-dependent signaling pathway. Treatment of acute mouse hippocampus slices with MC4R agonist abolished the amyloid-beta-induced impairment in cAMP/PKA signaling and synaptic plasticity. Hence, our findings collectively indicate that MC4R activation alleviates the amyloid-beta-induced synaptic deficit in hippocampal neurons in a cAMP/PKA-dependent manner. Further mechanistic studies of the causative link between MC4R/cAMP/PKA signaling with synaptic dysfunctions observed in AD may provide insights into the potential development of MC4R as a molecular target of the disease.

Disclosures: **F. Gong:** None. **Y. Shen:** None. **M. Tian:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Support: HKUST661111

HKUST16102715

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SH Ho Foundation

MSD R&D China Postdoctoral Research Fellowship

Title: Understanding the melanocortin microcircuit in the mouse hippocampus

Authors: ***Y. SHEN**[1,2,3], M. TIAN[1,2,3], F. GONG[1,2,3], Y. ZHENG[1,2,3], A. K. FU[1,2,3], N. Y. IP[1,2,3]; [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., [1]The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Hippocampal synaptic plasticity is the major cellular mechanism underlying learning and memory. Dysfunction or failure of hippocampal synaptic plasticity is associated with the development of various neurological disorders such as Alzheimer's disease. Hence, studying the functional regulation of synaptic plasticity not only enhances our understanding of learning and memory, but also elucidates the pathophysiology of dementia-related neurodegenerative diseases. Interestingly, melanocortins, the pro-opiomelanocortin (POMC)-derived peptides originally shown to regulate energy metabolism, are implicated in learning and memory enhancement. Here, we demonstrated that melanocortin 4 receptor (MC4R) plays an important role in the regulation of hippocampal synaptic plasticity. Long-term potentiation in the hippocampal CA1 region was significantly enhanced by *in vivo* MC4R activation and attenuated by MC4R knockdown. To further study the circuitry and mechanisms underlying melanocortins and MCRs involved in hippocampal synaptic plasticity, we mapped the circuitry of POMC/MCRs in the mouse hippocampus. We also confirmed melanocortin secretion in the hippocampus upon activity stimulation. These findings help to elucidate the hippocampal circuitry involved in learning and memory as well as the functions of the melanocortin system in the mammalian brain. Furthermore, the findings reveal a functional association between brain metabolism and cognition.

Disclosures: **Y. Shen:** None. **M. Tian:** None. **F. Gong:** None. **Y. Zheng:** None. **A.K. Fu:** None. **N.Y. Ip:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HI11C1186

HI12C0983

2011-0021866

Title: Dysregulation of microRNA188 expression causes cognitive impairments in Alzheimer's disease by inducing synaptic deficit

Authors: *H. KIM[1], K. LEE[1], K. AN[2], O.-B. KWON[2], S. PARK[2], J. CHA[1], M.-H. KIM[1], Y. LEE[2], J.-H. KIM[2], K. CHO[3], H.-S. KIM[1];
[1]Seoul Natl. Univ., Yeongeon-Dong, Jongno-Gu, Seoul, Korea, Republic of; [2]POSTECH, Pohang, Korea, Republic of; [3]Univ. of Bristol, Bristol, United Kingdom

Abstract: MicroRNAs have emerged as key factors in development, neurogenesis and synaptic functions in the central nervous system. In the present study, we investigated a pathophysiological significance of microRNA-188 (miR-188) in Alzheimer's disease (AD). We found that $A\beta_{1-42}$ treatment diminished miR-188-5p expression in primary hippocampal neurons and that miR-188 rescued $A\beta_{1-42}$-mediated synapse elimination and synaptic dysfunctions. Moreover, the impairments in cognitive function and synaptic transmission observed in 7-month-old 5Xfamilial AD (5XFAD) transgenic mice, were ameliorated via viral-mediated expression of miR-188. miR-188-5p expression was down-regulated in the brain tissues from AD patients and 5XFAD mice. The addition of miR-188 rescued the reduction in dendritic spine density in the primary hippocampal neurons-treated with oligomeric $A\beta_{1-42}$ and cultured from 5XFAD mice. The reduction in the frequency of mEPSCs was also restored by addition of miR-188. The impairments in basal fEPSPs and cognition observed in 7-month-old 5XFAD mice were ameliorated via the viral-mediated expression of miR-188 in the hippocampus. Furthermore, we found that miR-188 expression is CREB-dependent. Taken together, our results suggest that dysregulation of miR-188 expression contributes to the pathogenesis of AD by inducing synaptic dysfunction and cognitive deficits associated with Aβ-mediated pathophysiology in the disease

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Program#/Poster#: 412.12/P10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS030549

Title: Synaptic alterations consistent with parvalbumin-expressing interneuron dysfunction in a novel mouse model of Alzheimer's disease

Authors: *L. CHEN[1], T. SAITO[3], T. C. SAIDO[3], I. MODY[1,2];
[1]MCIP Interdepartmental Ph.D. Program, [2]Dept. of Neurology, David Geffen Sch. of Med., UCLA, Los Angeles, CA; [3]Lab. for Proteolytic Neurosci., RIKEN Brain Sci. Inst., Wako-shi, Japan

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that has become a compelling global public health concern. Besides pathological hallmarks such as extracellular amyloid plaques, intracellular neurofibrillary tangles, and loss of neurons and synapses, clinical reports have also shown that epileptiform activity, even seizures, can occur early in the disease, particularly in familial AD. Moreover, such hypersynchronous network activity might also be an integral part of early pathological events in sporadic AD. Therefore, studying the mechanisms underlying AD related network dysfunction and its relation to cognitive decline is an important aspect in AD research. The APP-NL/F-KI novel mouse model of AD has been generated by a gene knock-in approach, and it most closely replicates the pathologies found in human AD when compared to other existing transgenic mouse models. In this new model, we have studied potential alterations in synaptic function in parietal cortex layer II/III pyramidal cells in acute brain slices. Our data from whole-cell recordings show that compared to age-matched control WT mice, in ~15-month-old APP-NL/F-KI homozygous mice: (1) the average amplitudes of both sEPSCs and sIPSCs were decreased in the pyramidal cells (sEPSC: 29.12 ± 1.50 pA vs. 22.70 ± 0.66 pA, $p<0.01$; sIPSC: 66.14 ± 6.12 pA vs. 39.64 ± 3.31 pA, $p<0.01$; n=10 vs. n=7; WT vs. homo); (2) particularly, the average amplitude of large sIPSC events (as identified through bimodal amplitude distributions) was smaller (133.90 ± 15.20 pA vs. 75.78 ± 11.07 pA; WT vs. homo; $p<0.05$); (3) the fraction of multi-event (≥ 2) bursts among the large sIPSC events was significantly lower (0.44 ± 0.03 vs. 0.31 ± 0.04; WT vs. homo; $p<0.05$). These alterations could indicate deficits in perisomatic-targeting PV-expressing interneurons (PV INs), which generate strong inhibition on their postsynaptic targets. Previous studies in the APP-overexpressing mouse model of AD have revealed diminished PV IN intrinsic excitability caused by reduced expression of voltage-gated sodium channel Nav1.1. Our present findings are consistent with this hypothesis, but further experiments will have to be done in the APP-NL/F-KI model to investigate the level of Nav1.1 expression in PV INs. Moreover, PV IN dysfunction could lead to abnormal gamma oscillations, which will disturb normal cortical network synchrony and lead to epileptiform activity and cognitive impairment in the transgenic animals. In summary, our findings on the altered synaptic activity in the novel mouse model of AD will help us understand the mechanisms underlying network hyperexcitability that is commonly found in this disorder.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R21NS072631-01A

California Department of Public Health

Center for the Neurobiology of Learning and Memory at the University of California, Irvine

Title: Synaptic zinc deficiency induces neuronal hyperexcitability and impairs adult neurogenesis in the hippocampus of ZnT3KO transgenic mice

Authors: *E. VOGLER[1], X. WANG[3], S. MICHALSKI[3], X. GAO[4], M. MAHAVONGTRAKUL[1], R. BOHANNAN[2], J. CHEN[4], J. A. BUSCIGLIO[1];
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Abstract: Zinc is enriched in the mossy fiber tract of the hippocampus, where it is transported into synaptic vesicles at glutamatergic synapses by the ZnT3 protein and released during excitatory neurotransmission. Research has demonstrated that zinc modulates learning and memory, synaptic plasticity, synaptogenesis and synapse maturity. Zinc also reduces neuronal excitability, enhances inhibitory neurotransmission, suppresses excitatory neurotransmission, and delays development of seizures in models of epilepsy. Release of zinc during synaptic activity is associated with seizures through observations that ZnT3 knockout mice (ZnT3KO), which lack and therefore do not release synaptic zinc, are susceptible to induced seizure activity. ZnT3KO mice also have age-dependent cognitive impairment and alterations in neurotrophic and synaptic proteins. These observations suggest that the lack of synaptic zinc may also impair neurogenesis in an age-dependent manner. In fact, neurogenesis is regulated in part by synaptic activity and cell signaling pathways that are altered in aged ZnT3KO.
We investigated the effects of the genetic removal of synaptic zinc on neuronal activity and adult neurogenesis in the hippocampus through EEG and through immunohistochemistry for markers of seizure activity, neural stem cell proliferation and mature neuron production in ZnT3KO transgenic mice. We found excessive neuronal spiking activity starting at 2 month of age in ZnT3KO mice and age-dependent increases in markers of seizure activity beginning at 6 months of age in ZnT3KO hippocampus. We also found that at 8 months of age ZnT3KO have impaired neural stem cell proliferation and mature neuron production in the hippocampus.
These results indicate that lack of synaptic zinc induces neuronal hyperactivity in young animals

which results in impaired hippocampal neurogenesis, a component of neuronal plasticity that is critical for normal learning and memory, by 6 months of age, offering a mechanistic explanation for the age-dependent cognitive impairment in ZnT3KO mice at 6 months. Future experiments investigating the effects of the suppression of neuronal hyperactivity on neurogenesis and cognition in ZnT3KO mice may lead to targets for therapeutic interventions in pathologies where the modulatory role of zinc on neurotransmission is impaired.

Disclosures: **E. Vogler:** None. **X. Wang:** None. **S. Michalski:** None. **X. Gao:** None. **M. Mahavongtrakul:** None. **R. Bohannan:** None. **J. Chen:** None. **J.A. Busciglio:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant UL1 TR001414

FRT Award

DOE GAANN P200A120165

Title: Abnormal increased basal EEG activity in the ZnT3KO mouse model of Alzheimer's Disease

Authors: ***M. MAHAVONGTRAKUL**, E. VOGLER, J. YAO, A. TRAN, R. F. STEVENSON, D. TRAN, J. BUSCIGLIO;
Univ. of California, Irvine, Irvine, CA

Abstract: Alzheimer's Disease (AD) patients have increased risk of nonconvulsive seizures; however, only recently has hyperexcitability become a major focus of AD research. Synaptic zinc, packaged into vesicles via the zinc T3 (ZnT3) transporter, is co-released with amyloid beta (Aβ) during neurotransmission and is implicated in both oligomer formation and modulation of excitatory neurotransmission. ZnT3KO mice exhibit age-dependent increases in markers of seizure activity, synaptic loss, neurodegeneration, and cognitive impairment, which can be reversed by chronic, but not acute, treatment with the antiepileptic drug Levetiracetam. Thus, the loss of zinc neuromodulation emerges as a key element in the development of hyperexcitability, leading to cognitive decline. We further investigated the role of zinc in this pathological process by examining EEG profiles in WT and ZnT3KO mice. Fifteen-month old ZnT3KO and WT mice underwent a novel method of EEG surgery, with subcranial electrodes placed over the medial

prefrontal cortex and parietal cortex. A Neurologger device was attached to each mouse to record EEG activity for 24-48 hours. The data were analyzed using custom MATLAB scripts which characterized the frequency and amplitude of the waveforms from the different recording electrodes, as well as the synchrony between each recording site. ZnT3KO mice exhibited basal hyperexcitability compared with their WT counterparts, as indicated by an increase in both frequency and amplitude of the EEG waveforms. These mice also exhibited hypersynchronous firing across all electrodes as indicated by a decrease in signal variance among all four recording electrodes and an increase in amplitude accompanying a decrease in frequency in the summated EEG trace. These results indicate that electrical discharge in ZnT3KO mice is abnormal. This is significant because chronic hyperexcitability may lead to maladaptive circuit plasticity, neurodegeneration, and cognitive impairment in both AD mouse models and AD patients. Ongoing studies are focusing on characterizing the imbalance of excitation and inhibition, as well as defining the onset of abnormal EEG activity in relation to the time point at which cognitive impairment becomes apparent in ZnT3KO mice.

Disclosures: **M. Mahavongtrakul:** None. **E. Vogler:** None. **J. Yao:** None. **A. Tran:** None. **R.F. Stevenson:** None. **D. Tran:** None. **J. Busciglio:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: INSPIRE Fellowship

WELCOME Trust-DBT India Alliance

Title: Modified synaptic transmission and short-term plasticity in Alzheimer’s disease

Authors: ***N. SINGH**, S. NADKARNI;
Biol., Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Alzheimer’s disease (AD) is a multifaceted catastrophic disease that involves multiple brain areas resulting in a range of debilitating symptoms that increase in severity over time. The precise molecular mechanisms that underlie the constellation of deficits are not yet known. Early symptoms of AD; the inability to navigate through space and loss of short term memory, suggest that the hippocampal formation is impacted first in the disease. Further, these cognitive deficits precede structural changes in the brain.

Mutations in presenilins 1 and 2 (PS1 and PS2) are implicated in Familial Alzheimer's Disease (FAD). These mutations disrupt calcium homeostasis in cytosol, however the precise mechanism is still not clear. In order to gain a mechanistic understanding of this high-dimensional disease, we focus our investigations on one of the earliest pathological signs, namely, perturbations to intracellular calcium signals within hippocampal synapses. These perturbations modify the plasticity of synapses that ultimately may have a causal link to deficits in cognition.
Our modeling study is carried out in a CA3-CA1 synapse reconstructed from electron microscope images. We simulate signaling pathways relevant to synaptic transmission and short-term plasticity in the presynaptic terminal. We propose that calcium dynamics associated with the endoplasmic reticulum (ER) in the presynaptic terminal accounts for the short-term plasticity profile of the synapse and is essential for normal function. Further, FAD related mutations that compromise the leak channel in the ER, lead to an overload of calcium in the ER, and cause an inordinate increase in cytosolic calcium flux via ryanodine receptors (RyR). The augmented calcium signaling can cause rapid depletion of neurotransmitter vesicles. The contrasting dynamics of increase in release rate of vesicles from a small pool of available vesicles, and, the slow time scales of vesicle recycling, leads to compromised short-term plasticity. Inositol triphosphate receptor (IP_3R) also contributes to cytosolic calcium concentration by releasing calcium from ER in response to certain ionositol triphosphate and calcium concentration. Separately, we also investigate how the changes in binding kinetics and opening rates of IP_3R associated with FAD related mutations in PS, modulate synaptic transmission and other downstream signaling cascades involved in plasticity.

Disclosures: **N. Singh:** None. **S. Nadkarni:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The National Science Foundation of China 31171018

The National Science Foundation of China 31171355

Natural Science Foundation of Guangdong Province S2011010003403

Title: Biochemical and functional deficits in olfactory epithelium and olfactory bulb of 3-5 month old APP/PS1 mice

Authors: *M. CHEN;
South China Normal Univ., Guangzhou, China

Abstract: Olfactory dysfunction is considered as early syndrome of Alzheimer's disease (AD), which occurred before the appearance of learning memory deficits. Therefore, olfactory memory pathway may be the early targets impaired during the onset and progress of AD. In the present study, we observed a significantly reduced electroolfactogram (EOG) amplitude and number of olfactory sensory neurons (OSNs) in the olfactory epithelium of 3-5 months APP/PS1 mice, an age the mice displaying impaired cookie finding ability, compared to wild type (WT) littermate. *In vivo* local field potential (LFP) recording in OB revealed enhanced gamma oscillation power and reduced theta oscillation power accompanied by increased GABA receptor protein levels in APP/PS1 compared to WT mice, indicating that the change of inhibitory GABA signaling may contribute, at least in part, to altered oscillatory activities in OB. The present study suggests that 3-5 months APP/PS1 mice already show impaired olfactory perception, which is earlier than the deposition of Aβ plagues, implying the early dysfunction in olfactory pathway could be concerned as a biomarker for early diagnosis of AD. **Key words:** AD, mature OSNs, $GABA_AR$, electroolfactogram, oscillation. **Acknowlegement:** This work was supported by grants from The National Science Foundation of China (31171018, 31171355), Natural Science Foundation of Guangdong Province (S2011010003403).

Disclosures: M. Chen: None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Neurological Foundation of New Zealand

New Zealand Health Research Council

Title: Tumor necrosis factor-α mediates LTP impairment in APP/PS1 mice

Authors: *A. SINGH, O. D. JONES, W. C. ABRAHAM;
Dept. of Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disease and the most common cause of dementia. Synaptic dysfunction and loss in the hippocampus and association cortices are early and characteristic features of AD, and correlated with the severity of cognitive decline. Long-

term potentiation (LTP), an important mechanism underlying learning and memory, is highly regulated by many factors, including the history of prior cell activity (i.e. metaplasticity). Previously we described heterosynaptic metaplasticity in the rat hippocampus in which strong high-frequency priming stimulation in stratum oriens inhibited subsequent LTP elicited in the stratum radiatum of area CA1, an effect mediated by the astrocyte network. Since LTP is inhibited in the APP/PS1 (APP695swe/PS1-dE9) mouse model of AD, we hypothesized that this effect is due to aberrant engagement of this inhibitory metaplasticity mechanism at baseline. To investigate this, acute hippocampal slices were prepared from APP/PS1 and B6C3 wild type mice (12 mo) and field excitatory postsynaptic potentials were recorded from area CA1 following stimulation of Schaffer collateral fibres. The magnitude of LTP was significantly reduced in control transgenic mice (124 $\pm$ 3%, N=7) compared to wild type controls (143 $\pm$ 3%, N=8; $p<0.05$). Priming (6x100 Hz trains) in stratum oriens caused inhibition of radiatum LTP in wild-type mice (126 $\pm$ 2%, N=8) to a level comparable to that of the transgenics (124 $\pm$ 3%, N=7). We hypothesised that release of the cytokine tumor necrosis factor-α (TNF-α) from non-neuronal cells, possibly astrocytes, mediates the inhibition of LTP and that blocking TNF-α would rescue LTP in both the wild-type primed and control transgenic conditions. Primed LTP in wild-type mice was indeed rescued (145 $\pm$ 2%, N=8; $p<0.05$) to control levels by TNF-α antibody preincubation (25 µg/ml). Similarly, the TNF-α antibody rescued non-primed LTP in the transgenic mice (139 $\pm$ 3%, N=7; $p<0.05$) to wild-type levels. These data support the hypothesis that TNF-α mediated inhibition of LTP is aberrantly engaged in AD mice and contributes to the impairments in LTP and cognition seen in this model.

Disclosures: **A. Singh:** None. **O.D. Jones:** None. **W.C. Abraham:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Alz Assoc NIRG-11-198378

Title: Stability of synaptic proteins in the frontal cortex in the preclinical phase of Alzheimer’s disease

Authors: *M. A. ANSARI[1], S. W. SCHEFF[2];
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Abstract: Although the precise etiology of Alzheimer's disease (AD) remains to be elucidated, numerous studies have supported the idea that synaptic dysfunction, in key brain regions, may be a seminal event leading to cognitive impairment. Our previous studies have reported a significant decline in synaptic number and their associated proteins in the hippocampus during the preclinical progression of the disease. The hippocampus is one of the earliest brain regions affected, and the loss of synaptic proteins strongly correlates with numerous markers of oxidative stress. As the disease progresses, oxidative stress increases in other brain regions such as frontal cortex (Brodmann area 9). In the early stages of the disease, there are significant changes in various enzymatic and non-enzymatic markers of oxidative stress in the frontal cortex. This study was designed to test whether or not levels of synaptic proteins are altered in the frontal cortex in the very early (preclinical) phase of AD similar to that observed in the hippocampus. We analyzed short post-mortem tissues obtained from different cohorts: (1) individuals with low or no AD-type pathology and no cognitive impairment (LP-NCI), (2) individuals with high AD-type pathology and no cognitive impairment (HP-NCI), and (3) individuals with amnestic mild cognitive impairment (aMCI). Possible changes in both pre-synaptic (synapsin-I and synaptophysin) and post-synaptic (drebrin and PSD-95) proteins were analyzed in all age matched subjects. The results showed a stability of synaptic proteins in the frontal cortex in the early stages of the disease. These results support the idea that the frontal cortex, although affected late in the disease progression, remains unaffected in the preclinical stage. The hippocampus may be more sensitive than other brain regions during the early stage, and future therapeutic interventions should concentrate on this area.

Disclosures: M.A. Ansari: None. **S.W. Scheff:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 412.19/Q5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant AG011385

Title: Formation and repair of DNA double-strand breaks in neurons: implications for Alzheimer's disease and related disorders

Authors: *M. D. EVANS[1,2], D. CHENG[1], L. MUCKE[1,2];
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Abstract: In *postmitotic* cells, the maintenance of genomic integrity is crucial to prevent the activation of cell death programs such as apoptosis. This is arguably most important for neurons, because the majority of these cells cannot be replaced during adulthood. We recently made the surprising discovery that the most severe form of DNA damage, double-strand breaks (DSBs), occurs in neurons of wildtype mice as a result of physiological and experimentally induced brain activity, for example, engagement of different brain regions during exploratory behavior. These activity-induced DSBs are repaired within hours. However, in human amyloid precursor protein (hAPP) transgenic mice from line J20, which simulate key aspects of Alzheimer's disease, the baseline levels of DSBs are increased, most likely due to deficient DSB repair involving depletion of the DNA repair protein breast cancer factor 1 (BRCA1). Independent lines of evidence suggest that activity-induced DSBs may be involved in the regulation of neuronal gene expression and the creation of neuronal diversity.
The mechanisms underlying the formation and repair of neuronal DSBs in health and disease are not fully understood. To facilitate the investigation of these mechanisms, we developed a neuronal culture assay that allows for rapid post-hoc imaging and unbiased quantification of DSBs in thousands of cells. We have coupled this technique with combinatorial immunocytochemistry, enabling the identification of DSBs in multiple cell types including dentate granule cells, CA pyramidal neurons and inhibitory neurons, as well as glial cells. Using this technique, we have confirmed that neuronal activation causes DSBs and identified baseline differences in the levels of activity-induced DSBs across different cell types. We also found that such DSBs, as defined by γH2A.X immunofluorescent punctae, are efficiently repaired within 3 hours. We are currently using this methodology to explore the role of DSBs and BRCA1 in neuronal functions and integrity.

Disclosures: M.D. Evans: None. **D. Cheng:** None. **L. Mucke:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 412.20/Q6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Society

Title: The role of Drp1 in AMPAR trafficking and Alzheimer's disease

Authors: *M. J. HEIMANN[1], C. S. BINDA[1], E. BRAKSATOR[2], C. GUO[1], K. A. WILKINSON[1], J. M. HENLEY[1];
[1]Dept. of Biochem., [2]Dept. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease in which mitochondrial and synaptic dysfunction, as well as aberrant α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking and synapse loss, are major factors in the manifestation and progression of the disease. It has been reported that dysregulation of the post-translational modification SUMOylation plays a role in the characteristic cognitive deficits observed in AD. Therefore we systematically analysed protein levels of the three Small Ubiquitin-like MOdifier paralogues SUMO1-3, SUMOylation pathway enzymes and known SUMO substrates in human AD *post mortem* brain tissue obtained from the South West Dementia Brain Bank by western blotting. We observed a decrease in protein level of the SUMO substrate dynamin-related protein 1 (Drp1) in Brodmann area 10 (BA10), a region in the prefrontal cortex severely affected by the disease, but not in cerebellum, a region largely spared by the disease, indicating a disease-specific reduction of Drp1. Drp1 is a mitochondrial GTPase required for fission and it plays an important role in synaptic function, structure and synaptic development. We then sought to examine the role of Drp1 and Drp1 SUMOylation in AMPAR trafficking and whether Drp1 reduction contributes to the synaptic dysfunction observed in AD. Lentivirus-mediated knockdown of endogenous Drp1 in primary cortical neurons followed by surface biotinylation and western blotting showed that loss of Drp1 leads to a significant decrease of surface, but not total, levels of the AMPA receptor subunits GluA1 and GluA2 under basal conditions. However, chemically induced long-term depression (chemLTD) via bath application of N-methyl-D-aspartate (NMDA) showed that Drp1 knockdown does not occlude the initial internalisation of surface GluA2 characteristic of chemLTD. These results suggest that Drp1 plays a role in AMPAR trafficking under basal conditions. We are now investigating whether the observed reduction of surface GluA1 and GluA2 is caused by synapse loss, a major correlate of the cognitive decline in AD, using electrophysiology and immunocytochemistry. Furthermore we are using a lentivirus-mediated knockdown and rescue approach to examine the role of Drp1 SUMOylation in mediating its effects on AMPAR trafficking. Together, our data indicate that Drp1 function is perturbed in AD, potentially contributing to the aberrant AMPAR trafficking and synaptic dysfunction observed in the disease, and highlight Drp1 as a possible therapeutic target.

Disclosures: M.J. Heimann: None. **C.S. Binda:** None. **E. Braksator:** None. **C. Guo:** None. **K.A. Wilkinson:** None. **J.M. Henley:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 412.21/Q7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ISAO

Title: Early life stress worsens cognitive performance, synaptic plasticity and Aβ levels in APP/PS1 mice

Authors: *S. L. LESUIS, B. A. C. E. VAN HOEK, P. J. LUCASSEN, H. J. KRUGERS;
Ctr. for Neurosci., Univ. Van Amsterdam, Amsterdam, Netherlands

Abstract: Ample evidence has indicated that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which controls circulating levels of glucocorticoid (GC) hormones, occurs early in Alzheimer's Disease (AD). Elevations in GCs have been reported to increase Aβ formation and augment tau phosphorylation and accumulation, consistent with the hypothesis that high levels of GCs, as found in AD, may play a central role in the development and progression of AD.
Lasting alterations in HPA axis responsiveness and GCs are, at least in part, determined by experiences during the early postnatal period. Early life stress (ELS) increases later HPA axis responsiveness and GC levels, which accompany cognitive decline. Therefore, in this study we investigated whether and how early life stress can accelerate the development of AD pathology. Male WT and APP/PS1 mice were housed with limited nesting and bedding material from PND 2-9 (ELS). HPA axis responsivity, Aβ levels, synaptic plasticity and cognitive performance were assessed in animals aged 4-6 months and 10-12 months.
We show that APP/PS1 mice exposed to ELS have a higher stress reactivity compared both to control-reared transgenic mice and wild type ELS mice. These effects were accompanied by *enhanced* levels of soluble Aβ-related, both in 6 and 12 months old mice. In ELS APP/PS1 mice, synaptic plasticity of the hippocampal CA1 area was strongly and persistently *increased* from an early age onwards. Finally, spatial memory performance as assessed in the Barnes Maze was *impaired* in 12 month old APP/PS1 mice that were exposed to ELS. WT animals exposed to ELS and transgenic mice showed reduced levels of conditioned fear in an auditory fear conditioning task. Interestingly, conditioned fear responses were *increased* in transgenic ELS mice.
These studies indicate that early life adversity determines stress-responsiveness, synaptic plasticity and cognitive performance in mice with a relevant mutation for AD. Further understanding of the mechanisms underlying cognitive alterations in APP/PS1 mice as a consequence of early life stress will yield valuable insights on how adverse events determine the progression of AD.

Disclosures: **S.L. Lesuis:** None. **B.A.C.E. Van Hoek:** None. **P.J. Lucassen:** None. **H.J. Krugers:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ERC Grant 340429

Title: Impact of EphA4 ablation on cognitive function and disease pathology in a mouse model of Alzheimer's disease

Authors: L. POPPE[1,2], L. RUÉ[1,2], Z. CALLAERTS-VEGH[3,4], R. D'HOOGE[5], R. LEMMENS[6,1,2], *W. L. ROBBERECHT[6,1,2];
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Abstract: Alzheimer's disease (AD) is the most common type of dementia, caused by degeneration of brain regions involved in memory and cognition. Early stages of the disease are characterized by synaptic dysfunctions and synapse loss, which correlate with the severity of memory loss in patients. So far, no effective cure for AD exists with current treatments mainly focusing on increasing the quality of life of patients. EphA4 is a tyrosine kinase receptor of the ephrin system mainly expressed in the hippocampus and cerebral cortex during adulthood. It is involved in the regulation of synaptic maintenance and plasticity. EphA4 activation results in downregulation of AMPA receptors, loss of synaptic glutamate transporters and retraction of dendritic spines. In amytrophic lateral sclerosis we demonstrated that EphA4 is involved in axonal regeneration capacity. Based on these various functions, we hypothesized that EphA4 ablation might mediate synaptic dysfunction and consequently cognitive performance in a mouse model of AD. We conditionally deleted EphA4 in the forebrain of APPPS1-21 mice. This mouse model is characterized by extensive amyloidosis of the brain, resulting in synaptic dysfunctions and cognitive deficits around the age of 9 months. To investigate the effect of EphA4 deletion on cognitive function, we performed a battery of behavioral tests, including the open field,

sociability/preference for social novelty, Morris water maze and contextual fear conditioning. Together, our results suggest an effect of EphA4 in ameliorating the cognitive deficits in this AD mouse model. Ongoing molecular analysis of amyloidosis and synaptic function will allow dissection of the underlying processes that have shown to improve cognitive defects.

Disclosures: **L. Poppe:** None. **L. Rué:** None. **Z. Callaerts-Vegh:** None. **R. D'Hooge:** None. **R. Lemmens:** None. **W.L. Robberecht:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Darwin Trust of Edinburgh

Euan MacDonald Centre for Motor Neurone Disease Research

BBSRC

Title: Combining molecular profiling of differentially vulnerable synaptic populations with in-vivo phenotypic assessment identifies regulators of neuronal stability

Authors: *M. LLAVERO HURTADO[1], H. R. FULLER[4], S. L. EATON[2], G. PENNETTA[3,5], J. D. COOPER[6], T. M. WISHART[2,5];
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Abstract: Synapses are an early pathological target in a wide range of neurodegenerative conditions including well known adult onset Alzheimer's, Parkinson's & Huntington's disease [1-3] and diseases of childhood such as the motor neurone disease - Spinal Muscular Atrophy and the neuronal ceroid lipofuscinoses (NCL's; A.K.A Batten disease) [4-6]. However, our understanding of the mechanisms regulating the stability of synapses and their exceptional vulnerability to neurodegenerative stimuli remains in its infancy.
To address this we are using the NCL's as a tool to identify novel regulators of synaptic stability, contributing to our understanding of a broad range of diseases and highlighting novel therapeutic

targets. The NCLs, are the most frequent autosomal-recessive disease of childhood [7]. There are currently 14 individual genes which mutations are capable of affecting lysosomal function, all of which result in similar phenotype including blindness, cognitive/motor deficits, seizures and premature death. Mutations in CLN3 underlie a juvenile form of NCL (JNCL), the most prevalent variant worldwide [8]. Differential vulnerability of distinct synaptic populations across different brain regions has been described in other models of NCL variants [5, 6] but not yet in JNCL. Here, we describe a similar pattern of synaptic loss in the Cln3 null mouse model of JNCL (*Cln3*$^{-/-}$). Secondly, we use this differential pattern of synaptic loss to map molecular expression profiles across three brain regions. Thirdly, this region vulnerability expression mapping revealed conserved molecular alterations between JNCL and other neurodegenerative conditions [9]. Finally, we demonstrate that genetic and/or pharmacological manipulation of candidate expression in *Drosophila* is sufficient to modulate disease progression *in-vivo*.

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Disclosures: M. Llavero Hurtado: None. **H.R. Fuller:** None. **S.L. Eaton:** None. **G. Pennetta:** None. **J.D. Cooper:** None. **T.M. Wishart:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 412.24/Q10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SUVN-G3031, a histamine 3 receptor inverse agonist potentiates the procognitive and neurochemical effects of donepezil and memantine

Authors: R. MEDAPATI[1], V. BENADE[2], S. DARIPELLI[2], G. AYYANKI[2], V. KAMUJU[2], R. ABRAHAM[1], P. JAYARAJAN[1], G. BHYRAPUNENI[2], K. BOJJA[3], *A. K. SHINDE[4], R. NIROGI[5];

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Abstract: Cholinesterase inhibitors and the N-methyl D-aspartate (NMDA) glutamate receptor inhibitor are the currently approved agents for the symptomatic treatment of dementia associated with Alzheimer's disease (AD). Memantine added to stable donepezil in AD patients is associated with significant benefits in reducing decline in cognition, function and global status. However, the improvement is modest. Blocking or negatively regulating the functions of histamine-3 (H3) receptor is currently being explored as a potential symptomatic treatment for AD. SUVN-G3031 is a potent and selective H3 receptor inverse agonist. We hypothesized that SUVN-G3031 may potentiate the therapeutic effects of memantine and donepezil. Hence, the effect of SUVN-G3031 in combination with memantine and donepezil was evaluated in object recognition task. The effect of the above combinations on the cholinergic neurotransmission was evaluated in the hippocampus of freely moving male Wistar rats using brain microdialysis and the effect on theta oscillation in hippocampus was measured using EEG in anesthetized rats. Co-treatment of SUVN-G3031 with memantine and donepezil significantly potentiated the procognitive effects when compared with memantine and donepezil treatment group. Similarly co-treatment of SUVN-G3031 with memantine and donepezil significantly enhanced the acetylcholine levels and theta oscillatory activity in the hippocampus. The enhanced procognitive effects seen in the group co-treated with SUVN-G3031, memantine and donepezil can be attributed to the augmentation of the cholinergic neurotransmission in the brain. Thus combination of SUVN-G3031 with memantine and donepezil may offer a new therapeutic strategy for the symptomatic treatment of Alzheimer's disease.

Disclosures: **R. Medapati:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Benade:** A. Employment/Salary (full or part-time): Suven Life Sciences. **S. Daripelli:** A. Employment/Salary (full or part-time): Suven Life Sciences. **G. Ayyanki:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Kamuju:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Abraham:** A. Employment/Salary (full or part-time): Suven Life Sciences. **P. Jayarajan:** A. Employment/Salary (full or part-time): Suven Life Sciences. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time): Suven Life Sciences. **K. Bojja:** A. Employment/Salary (full or part-time): Suven Life Sciences. **A.K. Shinde:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences.

Poster

412. Synaptic Pathology in Alzheimer's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TATA Trusts, India

Council of Scientific & Industrial Research, India

Title: Grx1 over-expression reverses spine loss in primary cortical neurons derived from Alzheimer's disease transgenic mice.

Authors: *D. DAS[1], R. P. KOMMADDI[1], A. RAY[1], B. L. SCHNEIDER[2], P. AEBISCHER[2], V. RAVINDRANATH[1];
[1]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India; [2]Neurodegenerative Studies Lab., Brain Mind Institute, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: $A\beta_{42}$, one of the major causative agents implicated in Alzheimer's disease (AD), is known to directly and indirectly generate reactive oxygen species (ROS) leading to oxidative stress. Perturbation of redox homeostasis contributes considerably to the pathogenesis of AD. This is presumably due to the fact that the brain is not well equipped with anti-oxidant defense mechanisms. Glutathione, the ubiquitous antioxidant, is present in smaller quantities in the brain compared to the periphery, rendering the brain more susceptible to oxidative stress. Oxidative stress adversely impact neuronal function by altering protein structure and function through mechanisms including sulfoxidation, glutathionylation, nitrosylation and carbonylation. Glutathionylation is a common downstream effect of oxidative stress, and glutaredoxin 1 (Grx1), a thiol disulfide oxidoreductase, catalyzes the reversal using reducing equivalents of glutathione. We used primary cortical cultures from the APP^{Swe}/PSEN1ΔE9 transgenic mice as an AD model system to study the role of oxidative stress in triggering structural changes in neurons. We measured changes in area of soma, total length and branching of neurites, and spine density. Interestingly, significantly higher levels of ROS were detected in both soma and neurites of transgenic neurons at 15 *days-in-vitro* (DIV). This corresponded with a significant loss in density of total and mushroom spines, starting at DIV-15, persisting upto DIV-21, while other morphological changes were only detected at DIV-21. This suggests that spine loss could potentially be one of the early structural changes downstream of oxidative stress. Mushroom spines are involved in formation of synapses, and a loss in these structures indicates functional impairments in neurons. In order to ascertain the contribution of ROS-dependent protein glutathionylation towards spine loss observed, we over-expressed Grx1 neurons and found that it rescued total and mushroom spine loss seen in these neurons. In conclusion, at least one of the mechanisms through which $A\beta_{42}$ triggers spine loss is via increased oxidative stress, resulting in glutathionylation of target proteins.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TATA trusts for Alzheimer's Research

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Title: Activity and levels of calpain 2 but not calpain 1 is upregulated in synaptosomes early in the pathogenesis of Alzheimer's disease

Authors: *F. AHMAD[1], D. BENNETT[2], V. RAVINDRANATH[1];
[1]Indian Inst. of Sci., Bangalore, India; [2]Rush Univ. Med. Ctr., Chicago, IL

Abstract: The pathogenic mechanisms underlying Alzheimer's disease (AD) are now known to include synaptic dysfunction even in early stages of AD when there is little β-amyloid deposition or neuronal death. While, synaptic dysfunction in AD is associated with multiple mechanisms including disrupted Ca^{2+} homeostasis, oxidative stress and mitochondrial dysfunction, the molecular players are largely unknown. Calpain, a calcium dependent, non-lysosomal cysteine protease plays a critical role in synapse function and plasticity. However, dysregulated global hyperactivation of calpain is implicated in neuronal death in various models of neurodegeneration, including AD models, late in the disease process. Since calpain plays an important role in normal synapse function, we sought to examine whether dysregulation of calpains contributes to early synaptic dysfunction in AD pathogenesis. To this end, we analyzed the expression profiles of two major brain isoforms of calpain; calpain 1 and 2 in synaptosomes and post nuclear supernatant (PNS) isolated from cortices of both young pre-symptomatic (1 and 3 months old) and middle aged symptomatic (9-12 months old) APPswe/PS1ΔE9 (APP/PS1) mice, a well established mouse model of AD. While expression of both calpain 1 and 2 were unaffected in PNS of APP/PS1 mice, we observed increased expression of calpain 2 in synaptosomes as early as 3 months of age that persisted up to 9-12 months of age. Importantly, activity of calpain 2 in synaptosomes was upregulated from 1 month onwards and persisted up to 9-12 months. Interestingly, calpain 1 expression was unaffected in synaptosomes of APP/PS1 mice at pre-symptomatic ages of 1 and 3 months of age and increased only at 9-11 months of age when overt behavioral and pathological symptoms are evident. Moreover, we observed increase in protein and activity levels of calpain 2 in synaptosomes isolated from post mortem frontal neocortical tissue of persons with AD compared to persons without cognitive impairment. Additionally, increase in synaptosomal calpain 2 activity significantly correlated with decline in

performance of in memory and cognitive tests as wells as increase in β-amyloid load. To our knowledge, this is the first study reporting synapse specific calpain 2 hyperactivation in APP/PS1 mouse model during an early presymtomatic age as well as in post mortem brains of persons with AD. This is interesting in view of findings from genetic studies confirming that calpain 2, but not calpain 1, is indispensible for synapse function and plasticity.

Disclosures: F. Ahmad: None. **D. Bennett:** None. **V. Ravindranath:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Science and Technology, India

TATA Trusts for Alzheimer's Research, India

Title: Structural changes in CA1 hippocampal neurons in adolescent Alzheimer's disease transgenic (APP^{Swe}/PSEN1ΔE9) mice.

Authors: *S. KUMAR, V. RAVINDRANATH;
Ctr. for Neurosci., Indian Inst. of Sci., Banaglore, India

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by decline in cognitive functions, including memory impairment. Synapse loss is considered to be an early event in AD pathogenesis. We examined spine density and structural changes in neuronal morphology in a mouse model of Alzheimer's disease using APP^{Swe}/PS1ΔE9 (APP/PS1) mice. Although spatial memory impairments are observed at 9 months of age in this mouse model, we hypothesized that structural changes including density of different subclasses of spines could precede these defects and arise early. Brains from 1 month and 18-21 months old APP/PS1 mice (wild-type and transgenic) were fixed for Golgi staining and 100 μm serial sections were cut for spine and morphometric analyses. The hippocampal CA1 region was chosen for this study. Interestingly, we observed for the first time tertiary apical dendritic spine loss, including reduction in mushroom spine density, as early as 1 month of age. This progresses during life-span extending to spine loss on secondary dendrites, seen as loss of mushroom and stubby spines, at 18-21 months of age. Interestingly, Sholl analysis also revealed limited morphological changes in apical arborization in 1 month APP/PS1 mice. These changes extended to both apical and basal arborization in 18-21 months old animals., Thus, our study

demonstrate that AD-relevant pathological changes affecting neuronal structural features start early and progress with increasing age, with spine loss as one of the earliest events.

Disclosures: **S. Kumar:** None. **V. Ravindranath:** None.

Poster

412. Synaptic Pathology in Alzheimer's Disease

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Program#/Poster#: 412.28/Q14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Histological evaluation of synaptic markers in animal models of alzheimer's disease

Authors: ***J. NEDDENS**[1], M. ZHAN[2], E. AUER[1], B. HUTTER-PAIER[1];
[1]Neuropharm., QPS Austria GmbH, Grambach, Austria; [2]Biomed. Sci., Univ. of Applied Sci., Graz, Austria

Abstract: Alzheimer's disease (AD) is an age-dependent neurodegenerative disorder that represents the most common dementia worldwide. The histopathological hallmarks of this disease are accumulation of ß-amyloid (Aß) both within neurons, and extracellular as senile plaques, and intracellular formation of neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau. These pathologies often affect synaptic function and can result in neuronal loss. Transgenic animal models are frequently used to test new compounds against AD and should thus mimic the most common disease pathologies. To investigate synaptic changes in different AD rodent models, we used two synaptic markers, synaptophysin and spinophilin, to quantify the synapse density in the hippocampus. synaptophysin represents a presynaptic protein, which is most commonly used to quantify synapses. By contrast, spinophilin a postsynaptic protein, which is specifically located on dendritic spines, is used to visualize spine density. Five different rodent models were analyzed: transgenic APPsi, TauP301L and double-transgenic APPsi x TauP301L rats as well as transgenic TBA2.1 and APP_{SL} mice. Our results show that hippocampal synaptophysin was significantly increased in all three transgenic rat models, while spinophilin levels did not change. APP_{SL} transgenic mice showed a progressive increase of synaptophysin in the hippocampal CA1 region while spinophilin was only increased at the age of 12 months. Further analyses of TBA2.1 mice did not result in any significant changes. Our results show that in most transgenic AD rodent models the presynaptic marker synaptophysin is strongly altered while the postsynaptic marker spinophilin is almost not changed, suggesting a disturbed synapse formation in transgenic AD rodent models.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Program#/Poster#: 412.29/R1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK-SPG2013-1

Title: Changes in the synaptic proteome in Alzheimer's disease indicate a role for ApoE4 in synapse degeneration

Authors: *R. J. JACKSON[1], M. LLAVERO[2], A. G. HERRMANN[1], C. M. HENSTRIDGE[1], D. J. LAMONT[3], T. M. WISHART[2], T. L. SPIRES-JONES[1];
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Abstract: Of the main pathological features of Alzheimer's disease (AD), synapse loss is the greatest correlate of clinical cognitive decline, and this synapse loss is thought to be central to disease pathogenesis. Although most cases of AD are not directly heritable, genetic risk factors have been identified, the strongest of which is the epsilon 4 isoform of Apolipoprotein E (ApoE4). The ApoE4 allele increases not only the chance of developing AD compared to the more common ApoE3 allele but also increases the rate of cognitive decline seen within the disease. The ApoE4 allele has been shown to affect the synaptic plasticity and cognition of APOE4 carriers without AD. However the mechanisms by which AD causes synaptic degeneration and the role that ApoE4 plays in that degeneration remains unclear.
Proteomic analysis of synaptoneurosomes isolated from 25 control and 30 Alzheimer's disease patients with known APOE genotypes have indicated that 241 proteins were changed in the AD synapse samples compared to control. IPA analysis of these proteins found that the top affected pathways were mitochondrial dysfunction, lipid metabolism and oxidative phosphorylation. A subset of proteins changed between control and AD were also found to be changed in the same direction when AD ApoE3 cases were compared with AD ApoE4 cases. The top affected pathways of this subset were lipid transport, ion binding and the immune system. This group of proteins included clusterin and complement C4, both of which also have been genetically linked to AD. Once validated by fluorescent western blot, these protein hits have been further investigated using array tomography in postmortem human brain tissue to assess their contribution to synapse loss.
This study reveals new proteins and pathways that may be involved in synapse degeneration in AD and highlights the role genetic risk factors in this process.

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Poster

412. Synaptic Pathology in Alzheimer's Disease

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Program#/Poster#: 412.30/R2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant 5P50AG005142-32

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Title: Processing bodies and oAB-induced synaptic mRNA dysregulation

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Abstract: Plasticity, memory encoding and consolidation are dependent on local protein synthesis, initiated and regulated at the synapse. Interactions of oligomeric ß-amyloid (oAβ) with synapses may modify mRNA targeting, transport and stability. Our previous results show that some mRNAs, translated at the synapse, are increased in early Alzheimer's Disease. One potential mechanisms is perturbation of intraneuronal granule-like droplets that sequester and stabilize mRNAs for degradation or translation, the processing bodies (PB's) and stress granules (SG's), respectively. We investigated effects of chronic, non-toxic doses of oAß on PB's and SG's in soma and dendrites of primary dissociated, or organotypic cultures of rat frontal cortex, and in 5xFAD transgenic mice. Cultures were prepared and incubated for 7 days in medium prior to addition of oAß or scrambled oAß (sAß) then maintained, with 3-4 day refeeding for 14 days. Cultures were harvested at 7 and 14 days and prepared for immunostaining or immunoblotting using antibodies for SG protein, elF3ß; PB protein, DDX6; and the mRNA-binding proteins FMRP and CPEB. Primary cultures were also probed for protein synthesis products using Click-iT® to reveal effects of oAβ on translation. Confocal imaging and analysis using ImageJ revealed a change in number, and in the size ratio of PB's in treated primary cultures. PB's were increased in somas and proximal dendrites of oAß-treated cultures after 7 days compared to control, whereas SG's were maintained at a low level in both. FMRP, a translational repressor, was depleted in neurites of treated cultures, whereas CPEB, a translation activator, appeared more abundant and present in puncta adjacent to sites of translation. However, oAβ did not

induce a significant increase in protein synthesis. Results from oAß-treated organotypic cultures also show enhanced PB droplet formation and concomitant increase in DDX6 protein after 7 and 14 days exposure. In addition, PB's were more widely distributed in cortical neuropil of 3 month old 5xFAD mice compared to controls. DDX6 is present in PB's containing stalled polysomes and thus an increase in DDX6 containing droplets in oAß treated cultures, and in 5xFAD mice, suggests that oAß may encourage mRNA silencing. However, as oAβ apparently fails to reduce protein synthesis in dendrites, a select group of mRNAs may be targeted to PB's whilst others are freely translated.

Disclosures: C. Williams: None. **S. Marquez-Vilendrer:** None. **M.A. Roof:** None. **S. Shetty:** None. **C.A. Miller:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Michael J Fox Foundation 11709

Title: Identification of drug candidates that block the effects of alpha-synuclein oligomers on membrane trafficking

Authors: C. SILKY[1], *R. YURKO[2], K. MOZZONI[1], C. REHAK[1], N. IZZO[1], G. RISHTON[1], G. LOOK[1], H. SAFFERSTEIN[1], S. M. CATALANO[1];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by dysfunction in motor control as well as non-motor symptoms and cognitive loss. While motor symptoms are currently addressed by therapeutics, cognitive deficits are not, and represent a huge unmet medical need for PD patients. These cognitive deficits may be caused by oligomeric forms of alpha-synuclein (Asyn) protein that inhibit SNARE mediated vesicle fusion (Choi '15), disrupt

synaptic plasticity and impair long-term potentiation (Diogenes ’12; Martin ‘12). We have developed a screening assay capable of measuring Asyn oligomer-induced changes in membrane trafficking rate in primary neuronal cultures 21DIV (z’ score= 0.91). This assay thus provides a disease-relevant measure of Asyn oligomer neuronal deficits related to the cognitive deficits observed in Parkinson’s disease, and can be used to identify potential therapeutics capable of blocking Asyn-induced trafficking deficits (Danzer ’09).
Oligomers of full length recombinant Asyn closely match those isolated from human PD patient brain (see accompanying abstract) and both human PD patient-derived and recombinant Asyn oligomers cause deficits in membrane trafficking rate. Preliminary screening of the NIH Small Molecule Repository has identified several compounds that reverse effect of recombinant Asyn oligomer by >80% while having no effect on membrane trafficking when dosed on their own, including FK-506, phenothiazine, and hyperoside. These compounds have been previously implicated in Parkinson disease research or relevant disease pathways (Perren et al. 2015; Ye et al. 2014; Zhang et al. 2014), validating the ability of this screening platform to identify PD-relevant therapeutic leads.

Disclosures: **C. Silky:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock. **R. Yurko:** A. Employment/Salary (full or part-time): CogRx. **K. Mozzoni:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock. **C. Rehak:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock. **N. Izzo:** A. Employment/Salary (full or part-time): Salary. **G. Rishton:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock. **G. Look:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **H. Safferstein:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock. **S.M. Catalano:** A. Employment/Salary (full or part-time): Salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

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Hope Center

Title: Structure of the N-terminal region of alpha-synuclein determines the rate of fibril growth

Authors: D. D. DHAVALE, C. TSAI, D. P. BAGCHI, L. A. ENGEL, J. SAREZKY, *P. T. KOTZBAUER;
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Abstract: The accumulation of alpha-synuclein (α-syn) fibrils is the defining pathologic process in Parkinson disease (PD). A pathogenic role for α-syn is supported by the identification of dominantly inherited α-syn (*SNCA*) gene mutations in rare familial PD. Fibril formation requires an initial nucleation event to produce seeds, followed by fibril growth, during which monomeric protein associates with existing seeds. To characterize fibril formation kinetics, we produced recombinant α-syn protein containing an N-terminal bicysteine tag (C2-α-syn), which enabled detection of both soluble oligomers and mature fibrils with fluorescein arsenical hairpin (FlAsH) dye. In seed growth by monomer association (SeGMA) assays measuring fibril growth in the presence of C2-α-syn monomer, some PD-associated α-syn mutations (H50Q and A53T) greatly increased growth rates, while other mutations (E46K, A30P and G51D) decreased growth rates. We also measured the growth of WT seeds extended by mutant monomer or mutant seeds extended by WT monomer, since interactions between mutant and WT α-syn proteins could also influence fibril accumulation in familial PD. We found that single amino acid differences between seed and monomer protein sequences consistently decrease growth rates. These results demonstrate that the rate of α-syn monomer association during fibril growth is highly dependent on alignment of the N-terminal region of α-syn polypeptide chains, and that only a subset of familial PD mutations causes fibril accumulation through increased fibril growth rates. SeGMA assays can be utilized to further elucidate structural requirements of fibril growth and to identify inhibitors of this process as a potential therapeutic approach in PD.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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USA National Institute of Health (ES22274)

LABEX BRAIN ANR-10-LABX-4

Title: HA53T-α-syn accumulation and associated neurotoxicity is prevented by the inhibition of mitochondrial fission

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[1]CNRS UMR 5293, Inst. des Maladies Neurodégénératives, Bordeaux, France; [2]Plymouth Univ., Plymouth, United Kingdom

Abstract: Post-mitotic cells such as neurons have to deal with toxic insults for the entire life span. The only way to survive is to maintain a really efficient clearance system to preserve the integrity of energetic balance. In Parkinson's disease (PD), genetic mutations can have detrimental effects on clearance machinery leading the accumulation of dangerous reactive species of which the most representative is α-synuclein (α-syn). The accumulation causes the impairment of mitochondrial functionality with the consequent drop in energy production inducing neuronal death. In this study we demonstrate the mutual relationship between α-syn accumulation and mitochondrial functions and the possibility to reduce neurodegeneration when acting on mitochondrial phenotype. Firstly we assessed the impact of the overexpression of the pathogenic human A53T-α-syn (hA53T-α-syn) on mitochondrial morphology. hA53T-α-syn-overexpressing neurons showed a shift of mitochondrial network toward a less elongated phenotype, associated with the impairment of mitochondrial functionality (membrane potential and spare respiratory capacity) both in vitro and in vivo. Confirming a previous work (Bourdenx et al., 2015), we observed neuronal loss in rats overexpressing the hA53T-α-syn in SNc. In addition we also found a significant accumulation of PK-resistant hA53T-α-syn aggregates. Based on these results we decided to evaluate the efficacy of a neuroprotective strategy aimed to modify the mitochondrial dynamics by reducing fragmentation. To that end, we used the small molecule mdivi-1 (Rappold et al., 2014), a selective inhibitor of the dynamin-related protein 1 (drp1), the major player in mitochondrial fission events. Mdivi-1 reestablished the normal mitochondrial phenotype and functionality both in cells and rats. Moreover the rescue of anormal mitochondrial morphology is followed by a significant neuroprotectection. Furthermore we observed a decrease inw PK-resistant and phosphorylated hA53T-α-syn in SNc. These results

suggest that the mitochondrial fate is tightly linked with the accumulation of the pathogenic forms of hA53T-α-syn and vice versa. A possible explanation is the generation of an amplification loop that begins with the translocation of drp1 on mitochondrial membrane caused by the accumulation of hA53T-α-syn (Guy et al., 2012). This is followed by the enhancement of mitochondrial fragmentation that in turn can have a worsening effect on cellular clearance machinery. The treatment with the fission inhibitor mdivi-1 is able to preserve the integrity of mitochondria thus breaking down the vicious circle.

Disclosures: **S. Bido:** None. **R. Fan:** None. **K. Tieu:** None. **E. Bezard:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant 1R01NS088533

Title: 14-3-3s reduce endogenous alpha-synuclein aggregation and toxicity induced by fibrillary alpha-synuclein

Authors: R. UNDERWOOD[1], B. WANG[1], *T. A. YACOUBIAN[2];
[1]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; [2]Dept Neurol, Univ. Alabama Birmingham, Birmingham, AL

Abstract: Alpha-synuclein (αsyn) plays a critical role in Parkinson's disease (PD). Recent research suggests a prion-like mode for αsyn toxicity: αsyn is released as aggregated species that cause further aggregation and toxicity in neighboring cells. We have been investigating the role of the 14-3-3 proteins in regulating αsyn propagation. 14-3-3s are chaperone-like proteins that reduce protein aggregation, regulate protein secretion, and promote cell survival. To examine the effect of 14-3-3s on αsyn propagation, we employed an αsyn fibril model. Treatment of primary hippocampal cultures with recombinant αsyn preformed fibrils has been shown to cause misfolding and aggregation of endogenous αsyn. Here we demonstrate that endogenous 14-3-3s are sequestered with S129-phosphorylated αsyn into insoluble aggregates: in PBS-treated cultures, immunocytochemistry using an antibody against all 14-3-3 isoforms shows diffuse staining throughout the cell body and neurites, but as early as seven days after treatment with αsyn fibrils, 14-3-3 staining colocalizes with S129-phosphorylated αsyn. Since 14-3-3s can regulate αsyn aggregation, we next tested whether overexpression of 14-3-3θ can reduce aggregation of endogenous αsyn upon αsyn fibril treatment in this model. Day *in vitro* (DIV5)

primary cultures from nontransgenic and transgenic mice that overexpress HA-tagged14-3-3θ under the Thy1.2 promoter were treated with PBS or αsyn fibrils at 0.5 or 1 μg/ml, and then stained for S129-phosphorylated αsyn at 7, 10, and 14 days after fibril treatment. We observed ~70% decrease in insoluble phospho-S129 αsyn staining at 7 days after treatment with both doses. This reduction persisted at 14 days after treatment, with a reduction in phospho-S129 αsyn staining by 71% with 0.5 μg/ml fibril dose and by 61% with 1 μg/ml fibril dose in 14-3-3θ cultures compared to cultures from nontransgenic littermates. In concert with reduced phospho-S129 staining in 14-3-3θ cultures, we also observed a reduction in neuron loss at 14 days after fibril treatment. We are now examining αsyn aggregation and toxicity in primary cultures from nontransgenic and transgenic mice expressing the pan 14-3-3 peptide inhibitor difopein. We have also initiated pilot studies *in vivo* in which PBS or αsyn fibrils were stereotactically injected into the cortex of nontransgenic or 14-3-3θ transgenic mice. Based on these findings, we conclude that 14-3-3θ can regulate the propagation of αsyn and may serve as a target for therapeutic intervention in Parkinson's disease.

Disclosures: R. Underwood: None. **B. Wang:** None. **T.A. Yacoubian:** Other; Talene Yacoubian declares that she has a US Patent #7,919,262 on the use of 14-3-3s in neurodegeneration..

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Parkinsonfonden

Kempestiftelserna

Hjärnfonden

Title: Structural elucidation of alpha-synuclein oligomers using a library of sequential antibodies

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[1]Dept. of Chem., Umea, Sweden; [2]Dept. of medical biochemistry and biophysics, Umea, Sweden

Abstract: Misfolding and aggregation of the presynaptic protein alpha-synuclein (asyn) into amyloid fibrils is associated with Parkinson's disease (PD). It is increasingly accepted that it is the prefibrillar forms of asyn, the oligomers, that are the most toxic specie of the aberrantly folded asyn. Structural information about the oligomers is however scarce due to their transient

nature and their tendencies to aggregate. Using a library of peptide antibodies, spanning the sequence of asyn, we can uncover regions in the oligomers that differ in exposure when comparing with the monomer and with the help of an oligomeric-specific antibody we are able to capture oligomers from complex samples. By comparing the pattern of exposure we can acquire a fingerprint of an oligomer and reveal structural differences between oligomers formed under different conditions. Understanding the structure of the oligomeric species is vital for the development of effective therapeutics against this toxic specie.

Disclosures: **L. Nilsson:** None. **T. Islam:** None. **A. Olofsson:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: DA034783

Title: Multimer-PAGE reveals shift from multimeric to monomeric α-synuclein in HEK293 cells overexpressing A53T mutant α-synuclein

Authors: *B. A. KILLINGER, D. YEDLAPUDI, A. MOSZCZYNSKA;
Dept. of Pharmaceut. Sciences, EACHPS, Wayne State Univ., Detroit, MI

Abstract: The aberrant aggregation of α-synuclein is a key factor in Parkinson’s disease (PD). In vivo, α-synuclein likely exists in equilibrium between a monomer and several multimers. Studies suggest that a deficit in α-synuclein multimer formation is an initiating factor for its aberrant aggregation. Several α-synuclein mutations, including A53T, may impair α-synuclein multimer formation. Recently we described an analytical technique, termed multimer-polyacrylamide gel electrophoresis (multimer-PAGE), which allows for determination of α-synuclein multimer stoichiometry in brain tissue lysates. However, it remains unclear how this technique could be applied to disease models. Here we used multimer-PAGE to describe α-synuclein multimer stoichiometry in HEK293 cells overexpressing A53T mutant α-synuclein. Results show that wildtype (WT) α-synuclein, when overexpressed, displays a slight shift from multimer to monomer. In contrast, A53T overexpressing cells show a marked shift from the α-synuclein multimer to monomer. The shift from multimer to monomer in A53T overexpressing cells was not due to differences in transfection efficacy. Through further validation of multimer-PAGE, we also showed that the measured ratios were constant regardless of protein input and that binding efficacy of α-synuclein during the protein transfer likely explains why protein input was directly

proportional to the observed ratio of multimer to monomer. In conclusion, multimer-PAGE confirmed the shift in α-synuclein multimer to monomer as a possible disease initiating step in PD.

Disclosures: **B.A. Killinger:** None. **D. Yedlapudi:** None. **A. Moszczynska:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Support: Chow Yuk Ho Technology Centre for Innovative Medicine (4903747)

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Title: A high throughput screening system for visualizing therapeutic drugs of neurodegenerative diseases

Authors: ***S. L. WALKER**[1], D. C. W. CHAN[2], V. C. T. MOK[3], W. H. YUNG[2], Y. KE[2]; [1]The Chinese Univ. of Hong Kong, Shatin, Hong Kong, Hong Kong; [2]Sch. of Biomed. Sci., [3]Dept. of Med. and Therapeut., The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract: Brain diseases including neurodegenerative disorders pose one of the major challenges to the health care system worldwide particularly in regions, such as Hong Kong, that are facing a rapidly aging population. Development of new, more effective drug treatments to cope with neural diseases is a burning issue. However, current drug screening systems and procedures, especially for those targeting brain diseases, have severe limitations. To identify novel therapeutics, we have designed an in vivo imaging system which integrates behavioral dynamics of zebrafish, microfluidic devices, OLEDs, and multiphoton imaging into a robust imaging platform. Utilizing a preliminary design of this system, we were able to capture larvae zebrafish in a transferable microfluidic device, position under a multi-photon microscope for in vivo calcium imaging in the presence of a red light stimulus. This method was able to statistically and reproducibly differentiate between epileptic and healthy, Parkinson's and healthy, and their respective treatments in zebrafish larvae. In vivo neural imaging provided an avenue for distinguishing differences between currently available therapeutic drugs with the potential of identifying newer drugs in the future.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Ri.MED Foundation Italy

Title: Novel reagents and assays indicate a role for NADPH oxidase 2 in Parkinson disease

Authors: *J. T. GREENAMYRE, E. K. HOFFMAN, M. T. KEENEY, J. MCCOY, P. J. PAGANO, R. DI MAIO;
Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Mitochondrial defects and oxidative stress have been strongly implicated in the pathogenesis of Parkinson disease (PD). While it is generally assumed that the oxidative stress and damage seen in PD derive from mitochondria, there is growing evidence that reactive oxygen species (ROS) generated by NADPH oxidase 2 (NOX2) may be important. Indeed, mitochondrial dysfunction and NOX2 are intimately related. While there is some evidence that NOX2 inhibitors may protect dopaminergic neurons against degeneration, these studies have often been hampered by the lack of highly specific inhibitors. Additionally, it has been difficult to assess the activation state of NOX2 under experimental or pathological conditions with a cellular level of resolution. We now report testing of a novel and highly specific NOX2 inhibitor, Nox2ds-tat, and development of a new histological assay for NOX2 activation that is based on association of NOX2 and $p47^{phox}$, which is required for activation, and detected by proximity ligation.

To model certain aspects of PD in vivo and in vitro, we used the mitochondrial complex I toxin, rotenone. In SH-SY5Y cells exposed to sublethal rotenone, there was clear-cut activation of NOX2. Similarly, in rotenone treated rats, there was strong activation of NOX2 in nigrostriatal dopamine neurons. Importantly, the PL assay also detected NOX2 activation in dopamine neurons in brains of patients with PD. Thus, NOX2 activation occurs in vulnerable neurons in PD and models thereof.

In cell culture and in vivo, rotenone causes first (i) accumulation, then (ii) oligomerization, and later, (iii) fibrillization/aggregation of α-synuclein. Nox2ds-tat is a peptide that blocks association of NOX2 and $p47^{phox}$, thereby preventing NOX2 activation. Co-treatment of cultures with rotenone and Nox2ds-tat prevented the rotenone-induced accumulation, oligomerization and aggregation of α-synuclein.

Oligomeric α-synuclein binds to TOM70, a mitochondrial receptor that binds proteins containing an internal mitochondrial targeting signal. To facilitate import of these proteins, TOM70 also interacts with Hsp70 to prevent misfolding/aggregation of proteins to be imported. Rotenone

disrupts the normal TOM70:Hsp70 interaction and this is prevented by co-treatment with Nox2ds-tat, presumably because it reduces rotenone-induced oligomerization of α-synuclein. Together, these results indicate (i) that NOX2 activation occurs in PD, (ii) that NOX2 activity contributes to α-synuclein pathology, and (iii) that NOX2 activity contributes to mitochondrial impairment.
Supported by Ri.MED Foundation, Italy

Disclosures: J.T. Greenamyre: None. **E.K. Hoffman:** None. **M.T. Keeney:** None. **J. McCoy:** None. **P.J. Pagano:** None. **R. Di Maio:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: Parkinson's Disease Foundation

American Parkinson's Disease Association

Merck Sharp & Dohme Corp.

Title: Inhibition of LRRK2 prevents rotenone induced reduction of glucocerebrosidase activity.

Authors: *E. N. ROCHA, E. HOFFMAN, R. DI MAIO, P. J. BARRETT, J. T. GREENAMYRE;
Pittsburgh Inst. for Neurodegenerative Dis., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Mutations in *LRRK2* are the most common monogenic cause of Parkinson disease (PD) and mutations in *GBA1* are the most common risk factor for the disease. Whether and how these 2 genes and their protein products interact physiologically is unknown. LRRK2 is a kinase that has been associated with several pathways including autophagy, and mutations in LRRK2 are associated with enhanced kinase activity. *GBA1* encodes a lysosomal hydrolase, glucocerebrosidase (GCase), and mutations are associated with loss of function and increased α-synuclein accumulation. Further, we previously reported a ~50% loss of GCase activity in substantia nigra of young idiopathic PD brains. In an effort to model aspects of PD in vitro and in vivo, we have employed the mitochondrial complex I toxin, rotenone. Recent data has suggested that rotenone causes an autophagy deficit; however, the effect of rotenone on GCase activity has not been explored. Rotenone also increases α-synuclein and, as reported elsewhere at this meeting, it can activate LRRK2 in vitro and in vivo. We now report that in cultured SH-

SY5Y cells, sublethal rotenone inhibits GCase activity by about 50% without causing overt toxicity or cell loss. In rotenone-treated rats, we have found a similar ~50% loss of GCase activity in substantia nigra - as seen in the human disease. GCase activity was unchanged in striatum and hippocampus. Interestingly, in the SH-SY5Y cells, co-treatment with the selective LRRK2 kinase inhibitor, GNE-7915 (50-500 nM), prevented the rotenone-induced loss of GCase activity. Our data suggest that mitochondrial complex I defects can alter lysosomal function, potentially reducing degradation of α-synuclein. Additionally, LRRK2 activity contributes to the impairment of GCase activity that is caused by mitochondrial dysfunction.

Disclosures: E.N. Rocha: None. **E. Hoffman:** None. **R. Di Maio:** None. **P.J. Barrett:** None. **J.T. Greenamyre:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Support: VA Merit Review (BX000552-06)

Title: Glatiramir acetate (Copaxone) causes restoration of the nigrostriatal pathway in a progressive MPTP mouse model of Parkinson's disease.

Authors: *C. K. MESHUL[1,2], M. J. CHURCHILL[1];
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Abstract: Patients with Parkinson's disease (PD) have been shown to have an increased inflammatory response as the disease progresses (Greer et al., 2008) and is associated with activation of the pro-inflammatory m1 subtypes of microglia. There are also m2 types of microglia that act as an anti-inflammatory. These m2 subtypes secrete growth and regenerative factors into the area of the injury (Olson and Grendelman, 2016). A shift from the pro-inflammatory microglia (m1) to the anti-inflammatory (m2) subtypes could be therapeutically relevant in PD by allowing the m2 subtypes to promote recovery of dopamine (DA) neurons and terminals. Yang et al. (2016) reported that after directly infusing IL-4 (an activator of m2 subtypes) into the area of an intracerebral hemorrhage, there was improvement in neurobehavioral tests. Glatiramir acetate (GA)(Copaxone), a current therapy for MS, is a known microglial activator that polarizes the m1 subtypes into m2. To test GA in a more clinically relevant neurorestoration model, we utilized a mouse model of PD by administration of MPTP

over a 4-wk time frame that results in a 50-70% loss of DA (Goldberg et al., 2011). GA treatment (1.5mg/kg or 3.5 mg/kg i.p) was initiated following (i.e., restoration) 4 weeks of MPTP administration. Grip test analysis revealed that the MPTP only group had a 50% increase in grip strength compared to the vehicle group (p=0.0001). This was attenuated in the MPTP+GA group, resulting in only a 12% increase in grip strength compared to the vehicle group (p=0.0004) **despite** the previous administration of MPTP. Optical density and protein analysis for tyrosine hydroxylase (TH) in both the striatum and substantia nigra (SN) revealed a 65% loss of TH in the MPTP only group compared to the vehicle group (p=0.0001). In comparison, there was **only** a 13% loss of TH in the MPTP+GA compared to the vehicle group despite 4 weeks of previous MPTP administration (p=0.0191). IBA1, a structural protein of microglia, was increased in the SN by 163% in the MPTP only group (p=0.0001). This increase was reversed in the MPTP+GA groups to the level of the vehicle group (p=0.46). This suggests that GA caused a decrease in either number or size of the microglia. Microglial markers typifying the two subtypes, nitric oxide synthase 2 (m1) and arginase 1 (m2) showed no changes, compared to either the vehicle or MPTP groups. Overall, our data suggests that GA caused a recovery of the TH within the remaining DA neurons of the SN, resulting in improved motor function and TH levels within the striatum. Interestingly, the microglia analysis suggests that GA results in neurorestoration through downregulation of microglia rather than polarizing the m1 subtypes into m2.

Disclosures: C.K. Meshul: None. **M.J. Churchill:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 413.11/R13

Topic: C.03. Parkinson’s Disease

Title: N-acylethanolamine acid amidase (NAAA) as a therapeutic target for Parkinson’s disease

Authors: *S. PONTIS[1], N. REALINI[1], F. PALESE[1], A. ARMIROTTI[1], M. LANFRANCO[1], D. PIOMELLI[1,2];
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Abstract: Abnormal innate immune responses and, in particular, microglia activation are thought to contribute to the pathogenesis of Parkinson’s disease (PD) (Holmans et al., 2013; Tansey and Goldberg, 2010). The lysosomal cysteine hydrolase, N-acylethanolamine acid amidase (NAAA), regulates immune responses in peripheral tissues by deactivating the fatty acid

ethanolamides, a family of anti-inflammatory lipid amides produced by macrophages and other host defense cells (LoVerme et al., 2005; Solorzano et al., 2009). Expression of NAAA in the healthy mouse brain is virtually undetectable (Tsuboi et al., 2007), but our preliminary studies show that it can be rapidly and persistently induced by intra-striatal administration of the neurotoxin 6-hydroxydopamine (6-OHDA). Immunofluorescence (IF) experiments show that 6-OHDA injections cause, within 48 h, a transient increase in NAAA expression in dopaminergic neurons (tyrosine hydroxylase positive, TH+) of the substantia nigra pars compacta (SNc), followed by a profound and long-lasting (2-3 weeks) NAAA induction in Iba-1+ microglia of striatum and SNc. To investigate the significance of these changes, we used mice that either overexpress NAAA in cells of monocyte lineage (CD11b+), including microglia (NAAA-ki mice), or constitutively lack NAAA expression (NAAA-ko mice). NAAA-ki mutants were obtained by crossing CD11b-Cre mice with heterozygous NAAA conditional knock-in mice in which the NAAA coding sequence was inserted within the Rosa26 locus. The animals are viable, fertile and display cell-appropriate NAAA overexpression. Compared to wild-type controls, NAAA-ki mice treated with intrastriatal 6-OHDA show substantially higher: (i) behavioral response to the dopamine receptor agonist apomorphine; (ii) TH+ neuron toxicity in the SNc (15.96±0.54% vs 26.78±0.36%; n=4); and (iii) mortality (47.8±4.8% vs 25.0±3.0%; n =20). The NAAA-ko mice [Naaatm1a(KOMP)Wtsi] were generated at the Welcome Trust Sanger Institute and obtained from Jackson Labs. The mice express a frame-shifted catalytically inactive protein, and are viable and fertile. Compared to wild-type mice, NAAA-ko mutants display significantly lower (i) behavioral responses to apomorphine (Fig. 1); (ii) TH+ neuron toxicity in the SNc (51.54±2.9% vs 26.78±0.36%; n =4); and (iii) mortality 16.6±5.3% vs 25.0±3.0%; n = 20). Collectively, the results suggest that NAAA is critically involved in the neuronal and microglial response to 6-OHDA, and that small-molecule agents targeting this enzyme may interrupt the pathological process initiated by the neurotoxin.

Disclosures: **S. Pontis:** None. **N. Realini:** None. **F. Palese:** None. **A. Armirotti:** None. **M. Lanfranco:** None. **D. Piomelli:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Program#/Poster#: 413.12/R14

Topic: C.03. Parkinson’s Disease

Support: NIH T32 AG023477

Title: The neurorestorative potential of cortical disinhibition in a progressive mouse model of Parkinson's disease

Authors: *R. HOOD[1], C. K. MESHULK[1,2];
[1]Behavioral Neuroscience, Oregon Hlth. & Sci. U, Portland, OR; [2]Portland VA Med. Ctr., Portland, OR

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder affecting 7-10 million people worldwide. Through a progressive loss of dopaminergic cells in the substantia nigra pars compacta (SNpc), signaling in the basal ganglia becomes dysfunctional, leading to declining motor and cognitive function. Targeting brain regions affected by PD may alleviate neurodegeneration that occurs in PD. Because motor cortex (MC) activation is altered and repetitive transcranial magnetic stimulation can temporarily attenuate symptoms in PD patients, we believe that the MC is a viable therapeutic target. We have shown that MC disinhibition through unilateral *Vgat* (the gene encoding the vesicular GABA transporter VGAT) knockout in the motor cortex of Vgatflox/flox mice is neuroprotective. Mice were stereotaxically injected with AAV-Cre-GFP unilaterally targeting the MC and then subjected to 4 weeks of an increasing dose of MPTP, 5 d/wk, injected i.p. at doses of 8, 16, 24, and 32 mg/kg/d. Since VGAT is required for GABAergic function, we believe targeting GABAergic interneurons with focal AAV-Cre-GFP prevents inhibitory GABAergic signaling in the MC. Control animals exhibit a 79% loss of tyrosine hydroxylase (TH) in the striatum after MPTP lesioning, and this loss was completely prevented by cortical disinhibition following AAV-Cre-GFP injection. Similarly, control animals showed a 59% loss of TH-expressing cells in the SNpc after MPTP lesioning and this loss was completely prevented by cortical disinhibition. However, this paradigm uses a neuroprotective design in which the therapy is administered before neurotoxic insult. While the results are promising, this study design is not clinically relevant. Therefore, we investigated the neurorestorative potential of cortical disinhibition in which mice were subjected to MPTP lesioning first and then immediately injected with either AAV-Cre-GFP or AAV-GFP unilaterally in the MC. Based on previous therapies tested with our model that were beneficial in both protective and restorative study designs, we hypothesized that cortical disinhibition will restore physiological or motor dysfunction observed after MPTP lesioning. Observing restoration in this study would suggest that cortical disinhibition could be an effective novel therapy for PD.

Disclosures: R. Hood: None. **C.K. Meshulk:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Grants from the National Natural Science Foundation of China (81373397)

The Jiangsu Department of Science and Technology Basic Research Program (No. BK20131443)

Title: Neuroprotective effects of α7 nicotinic acetylcholine receptor through the Wnt/β-catenin signaling pathway in both *In vivo* and *In vitro* models of Parkinson's disease.

Authors: *H. JUN[1], Y. FAN[2];
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Abstract: A growing body of evidence indicates that α7 nicotinic acetylcholine receptors (α7-nAChRs) play an important role in chronic inflammatory and neurodegenerative disease. However, the molecular mechanisms are still unknown. The objectives of the present study were to explore the potential of the α7-nAChRs activation for the treatment of Parkinson's disease (PD) and to determine whether its neuroprotective effects are exerted through the Wnt/β-catenin signaling pathway by using in vivo and in vitro models of PD. In the *in vivo* study, the α7 mutant mice (KO) aggravated the behavioral deficits of "Pole test", and dopaminergic cell loss that were induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a dose-dependent manner in an in vivo model of PD. In the *in vitro* study, cell damage induced by 1-methyl-4-phenylpyridinium (MPP+) was ameliorated by nicotine pretreatment. Nicotine induced protective effects on the protein and mRNA expression levels of markers of the Wnt/β-catenin signaling pathway in both the *in vivo* and the *in vitro* studies, and these neuroprotective effects were abolished by the α7 KO mice in the *in vivo* study or the α7-nAChR-selective antagonist in the *in vitro* study. Our results provide evidence that activation of α7-nAChRs has neuroprotective effects in both *in vivo* and *in vitro* PD models, and these effects act through the Wnt/β-catenin signaling pathway. Taken together, these results indicate that α7-nAChR agonist may exert therapeutic effects on PD via the Wnt/β-catenin signaling pathway and may therefore provide a novel approach for the treatment of PD.

Disclosures: H. Jun: None. **Y. Fan:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 413.14/R16

Topic: C.03. Parkinson's Disease

Title: Neuroprotective effect of dimethyl fumarate in mptp-mouse model of parkinson's disease

Authors: *E. ESPOSITO, F. BIUNDO, G. CASILI, M. CAMPOLO, R. CRUPI, M. CORDARO, S. CUZZOCREA;
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Abstract: Oxidative stress is central in Parkinson's disease (PD) and nuclear transcription factor related to NF-E2 (Nrf-2) is involved in neuroprotection against PD. The aim of the present study was to investigate the neurotherapeutic action, Nrf-2 dependent, of DMF in a mouse model of PD. Mice received four injections of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Starting 24 h after the first administration of MPTP, animals were treated with DMF (10, 30 and 100 mg/kg, by oral gavage) daily for 7 days and, on the 8th day mice were subjected to behavioural test. DMF treatment significantly reduced neuronal degeneration of dopaminergic tract and behavioral impairments induced by MPTP administration. Moreover, treatment with DMF prevented dopamine depletion increasing tyrosine hydroxylase (TH) and dopamine transporter (DAT) and also reduced α-synuclein-positive neurons. Furthermore, DMF treatment up-regulated Nrf-2 pathway, increasing $NeuN^{+}$/$Nrf\text{-}2^{+}$ cells in the striatum and inducing activation of manganese *superoxide dismutase* (Mn-SOD) and heme-oxygenase-1 (HO-1). Also, DMF reduced ciclo oxygenase 2 (COX-2), lowered nitrotyrosine (NT) and neuronal nitrite oxide synthase (nNOS) expression, restored *nerve growth factor* (*NGF*) levels and preserved by microtubule-associated protein 2 (MAP-2) alterations. These results support the thesis that DMF may constitute a promising therapeutic target for the treatment of PD.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 413.15/R17

Topic: C.03. Parkinson's Disease

Title: Recovery of motor functions in a Parkinson's disease rat model after releasing dopamine from titanium dioxide matrix implants

Authors: *M. GÓMEZ-CHAVARÍN[1,2], P. PADILLA[1], J. RAMIREZ-SANTOS[1], G. PRADO-PRONE[3], J. GARCÍA-MACEDO[3], G. GUTIERREZ-OSPINA[1];
[1]UNAM-Biomedical Res. Institutute, Mexico D. F., Mexico; [2]UNAM-Medicine Fac., Mexico, Mexico; [3]Solid State Physics, UNAM-Physics Inst., Mexico, Mexico

Abstract: Motor symptoms of Parkinson's disease (PD) are in part due to the depletion of dopamine (DA) in the caudate nucleus (CN). Insufficient availability of DA follows the loss of dopaminergic neurons in the substantia nigra (SN). Treatment with L-DOPA, a precursor of dopamine, partly restores motor function in Parkinson's disease patients. This treatment, however, requires the remaining nigral dopaminergic neurons to be able to synthesize dopamine. Since these neurons die progressively as the disease advances, L-DOPA becomes increasingly ineffective in attenuating motor symptoms in patients. It is then needed to develop a way to provide DA to the CN. Unfortunately, DA is oxidized rapidly after being released, a process that renders it biologically inactive if provided chronically with no protection. Hence, in this work we tested a semi-solid matrix made of titanium dioxide (TiO_2) as a mean to release chronically DA into the CN while avoiding its oxidation. We then implanted TiO_2 matrices with or with out DA in the CN of adult male Wistar rats exposed to rotenone (1mg/kg during 21 days), an herbicide that induces PD-like motor symptoms in rodents. Recovery of motor functions was evidenced through motor coordination tests. Histological surveys were also carried out. Before implantation, DA loaded matrices were subjected to infrared spectroscopy, high resolution transmission electron microscopy, X-ray diffraction and HPLC analyses to establish the molecular structure of the lattice and to evaluate its ability to carry, protect and release DA. Once corroborated their physicochemical properties, the matrix was stereotaxically implanted in the dorsal CN and the rats were evaluated one and two months later. Our results show that ROT-exposed display significant deficits of motor coordination as compared to control rats. Such deficits were reversed in most ROT-exposed rats implanted with matrices releasing DA, but not in those implanted with unloaded matrices. The histological procedures revealed implants to be correctly allocated and showed increments of tyrosine hydroxylase immune-reactivity in the *substantia nigra* and CN ipsilateral to the implanted matrix. Tyrosine hydroxylase immune-positive cells were also observed in the subventricular zone. Together our results support that TiO_2 manufactured matrices constitutes an effective device to release chronically biologically active DA into DA-depleted CN.

Disclosures: M. Gómez-Chavarín: None. **P. Padilla:** None. **J. Ramirez-Santos:** None. **G. Prado-Prone:** None. **J. García-Macedo:** None. **G. Gutierrez-Ospina:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Program#/Poster#: 413.16/S1

Topic: C.03. Parkinson's Disease

Support: Focused Ultrasound Foundation

Title: Long-term transgene expression in rat brain after intranasal administration of hGDNF DNA nanoparticles and enhancement by focused ultrasound (FUS)

Authors: *A. E.-E. ALY[1], T. SUN[2], Y. ZHANG[2], O. SESENOGLU-LAIRD[3], L. PADEGIMAS[3], M. J. COOPER[3], N. J. MCDANNOLD[2], B. L. WASZCZAK[1];
[1]Pharmaceut. Sci., Northeastern Univ., Boston, MA; [2]Radiology, Harvard Med. Sch. and Brigham & Women's Hosp., Boston, MA; [3]Copernicus Therapeut., Cleveland, OH

Abstract: The therapeutic potential of glial cell-line derived neurotrophic factor (GDNF) for treating Parkinson's disease (PD) has been limited thus far by its inability to cross the blood-brain barrier (BBB). We have previously shown that intranasal administration of PEGylated lysine 30-mer (CK30PEG10K) DNA nanoparticles (NPs) encoding hGDNF, developed by Copernicus Therapeutics, Inc., can transfect brain cells in vivo, induce transgene expression, and provide neuroprotection of substantia nigra (SN) dopamine neurons in the rat 6-hydroxydopamine model of PD. We have also shown using double-label immunohistochemistry that transgene expression in the rat brain occurs primarily in cells lining the vasculature, most likely pericytes.
The first goal of our current study was to assess the duration of eGFP expression after intranasal administration of DNA NPs which encode an eGFP-GDNF fusion protein. Rats were sacrificed 1 week, 3 months, or 6 months after intranasal administration. Analysis of eGFP expression at the 3 time points revealed that transgene expression was highest at one week, and persisted at ~30% of maximal levels at both the 3 and 6 month time points.
Although intranasal administration allows large biomolecules to bypass the BBB, it is a low efficiency route of administration and results in widespread distribution with no means of targeting specific brain regions. Focused ultrasound (FUS) combined with a circulating microbubble agent has been shown to enhance delivery of macromolecules to the brain by transiently disrupting the BBB. Our second goal was to evaluate whether FUS can be combined with intranasal administration of our DNA NPs, as well as the naked plasmids, to yield greater overall delivery, improved tissue penetration, and enrichment of transgene expression in the desired location(s) in brain. Results from a pilot study in 3 rats in which the naked plasmid was administered intranasally in conjunction with FUS and microbubbles provided evidence that the amount of transgene expression was increased on the sonicated versus the unsonicated side of the

brain, and overall whole brain eGFP weighted averages were significantly increased in the sonicated rats. Ongoing studies will examine the effect of FUS on transgene expression in the sonicated regions, and will assess tissue penetration and cell types transfected after intranasal administration of our DNA NPs.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 413.17/S2

Topic: C.03. Parkinson’s Disease

Title: Metabolic profiling and lipidomics of a potential canine model for a juvenile Parkison disease.

Authors: ***T. L. KOZICZ**[1,2], T. EMMERZAAL[1], F. VAZ[3], G. S. JOHNSON[4], D. P. O’BRIEN[5], C. P. BAINES[6], E. MORAVA[2];
[1]Anat., Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands; [2]Hayward Genet. Ctr., Tulane Univ., New Orleans, LA; [3]Lab. Genet. Metabolic Dis., Academic Med. Ctr., Amsterdam, Netherlands; [4]Dept. of Vet. Pathology, [5]4Department of Vet. Med. and Surgery, [6]Dalton Cardiovasc. Res. Ctr., Univ. of Missouri Col. of Vet. Med., Columbia, MO

Abstract: We evaluated a canine model with a homozygous splice donor mutation in the *SERAC1* gene as a potential animal model for MEGDEL syndrome and juvenile Parkinson disease. Metabolic profiling, lipidomics, phenotyping and imaging showed almost full overlap with those of patients with MEGDEL syndrome, including significant mitochondrial dysfunction of various complexes of the electron transport chain in a brain area specific manner as well as elevated phosphatidylglycerol-34:1 to phosphatidylglycerol-36:1 ratio. The neurologic phenotype showed early ataxia and later hypokinesia and postural instability. Brain

investigations detected cerebellar atrophy and basal ganglion involvement similar to juvenile Parkinson disease.
Our studies show novel and significant metabolic consequences in the *SERAC1* deficient brain, including abnormal cardiolipin synthesis and OXPHOS complex deficiencies. *SERAC1* is a complex lipid disorder and a new member of this evolving novel group of inborn errors of metabolism. As with several other complex lipid synthesis disorders, it has a presentation of juvenile Parkinson disease. The canine *SERAC1* mutant can be used as an animal model in future therapeutic trials in phospholipid related ataxia/dystonia.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Program#/Poster#: 413.18/S3

Topic: C.03. Parkinson's Disease

Support: CIHR

CFI

FRQS

Title: Therapeutic repurposing of raloxifene as an immunomodulatory drug for the treatment of gut inflammation in a mouse model of Parkinson's disease

Authors: *A.-A. POIRIER[1,2], M. CÔTÉ[1,3], M. BOURQUE[1,2], M. MORISSETTE[1], T. DI PAOLO[1,2], D. SOULET[1,3];
[1]Ctr. De Recherche Du CHU De Québec (CHUL), Quebec, QC, Canada; [2]Faculté de Pharmacie, [3]Faculté de médecine, psychiatrie et neuroscience, Univ. Laval, Québec, QC, Canada

Abstract: Motor symptoms in Parkinson's disease (PD) are often preceded by non-motor symptoms related to dysfunctions of the autonomic nervous system such as constipation, defecatory problems and delayed gastric emptying. These gastrointestinal disorders are associated with the alteration of dopaminergic (DA) neurons in the myenteric plexus (MP) of the gut. Studies in our laboratory have already demonstrated the immunomodulatory effect of female sex hormones to treat neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of enteric nervous system degeneration in PD and the implication of the G protein-coupled estrogen receptor 1 (GPER1). The objective of this project was to evaluate the

neuroprotective and immunomodulatory role of raloxifene, a selective estrogen receptor modulator already used for osteoporosis treatment. Adult C57BL/6 male mice received, daily for ten days, 2 injections of raloxifene (62.5 g) and/or G15 (10 or 50 g), a specific antagonist of GPER1. On day five, 4 injections of saline or MPTP (4.75 mg/kg) were administered. On day ten, mice were killed, the ileum was fixed and microdissected to isolate the MP. Cuprolinic blue staining and immunohistochemistry with antibodies against tyrosine hydroxylase (TH) and ionized calcium-binding adapter molecule 1 (Iba-1) were performed for stereological counting of total neurons, DA neurons (TH^+) and macrophages ($Iba\text{-}1^+$). We measured a loss of about 85% of TH^+ neurons in MPTP mice while control levels were maintained following raloxifene treatment. No difference in total neurons counts between groups was observed, suggesting that neuronal loss was specific to TH^+ neurons. Moreover, we observed an increase of approximately 55% in the number of macrophages in MPTP mice, while control levels were maintained with raloxifene treatment, demonstrating a significant anti-inflammatory effect of the drug in MPTP animals. When administered along with the antagonist G15, raloxifene did not lower the number of macrophages, indicating the important implication of GPER1. In addition, neurotoxin-induced proinflammatory polarization of human monocytic THP-1 cells, nuclear factor kappa B response and nitric oxide and proinflammatory cytokines production were also prevented by raloxifene *in vitro*. Overall, the present results demonstrate that raloxifene treatment prevent damages to DA neurons in the MP in a MPTP mouse model of Parkinson's disease, mainly through anti-inflammatory effects.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Financial support for this study was provided to the University of Kentucky by Alnylam Pharmaceuticals Inc. (Cambridge, MA) with funding received from the Michael J. Fox Foundation for Parkinson's Research via a competitive LEAPS award.

The hardware and software associated with the delivery system was provided by Medtronic Inc. (Minneapolis, MN).

Title: Tolerability of siRNA-mediated alpha-synuclein suppression in the adult rhesus substantia nigra

Authors: *R. GRONDIN[1], Y. AI[1], P. HUETTL[1], H. MENG[3], F. POMERLEAU[1], J. E. QUINTERO[1], P. A. HARDY[2], M. T. BUTT[4], A. SEHGAL[5], D. A. BUMCROT[5], D. M. GASH[1], Z. ZHANG[1], G. A. GERHARDT[1];
[1]Anat. & Neurobio., [2]Radiology, Univ. of Kentucky Med. Ctr., Lexington, KY; [3]Res. Ctr. for Sectional and Imaging Anat., Shandong Univ. Sch. of Med., Jinan, Shandong, China; [4]Tox Path Specialists LLC, Frederick, MD; [5]Alnylam Pharmaceuticals Inc., Cambridge, MA

Abstract: The histopathological hallmarks of Parkinson's disease (PD) include dopamine cell loss in the midbrain substantia nigra pars compacta and the formation of alpha-synuclein-rich intraneuronal inclusions, called Lewy bodies, in surviving neurons. Prior studies support a role for alpha-synuclein (α-Syn) in the pathogenesis of PD. Thus, approaches aimed at reducing the expression of α-Syn in the substantia nigra using small interfering RNAs (siRNAs) may be therapeutic. Whether prolonged suppression of wildtype α-Syn expression can be well tolerated in the adult brain is unclear. Here, we locally delivered varying concentrations of an siRNA construct directed against α-Syn into the subtantia nigra of 18 adult female rhesus macaques (n= 6 per concentration) at an infusion rate of 0.1 µL/min for 28 days using a pump and catheter system. Tolerability of prolonged α-Syn inhibition was assessed by clinical observations as well as by neurochemical and histopathological examinations of the striatum and midbrain regions. Histopathological examination of preserved tissues was performed by a board-certified veterinary pathologist with specific expertise evaluating the nervous system. Histopathological evaluations indicated that the catheter tip was placed in or near the subtantia nigra pars compacta region in all animals. Molecular analyses of mRNA levels from midbrain tissue punches indicated significant silencing of α-Syn expression ranging from 88.3±3.2% at a concentration of 6 mg/mL to 96.6±0.9% at a concentration of 18 mg/mL versus controls. There were no indications of toxicity as noted in clinical observations and body weight values. There were no test article-related changes in striatal dopamine content and no microscopic changes in the cerebrum/neocortex, ventricular system or limbic system/hippocampus. Microscopic changes in the midbrain were not related to the test article, but attributed to catheter placement including slight/minimal bleeding, astrocytic and microglial cell reaction, extracellular fluid and local tissue loss. There was no evidence of necrotic neurons in any of the midbrain sections evaluated. The microscopic changes noted did not result in clinical signs. Overall, our data support that targeted, intranigral delivery of siRNAs designed to suppress α-Syn expression should be further evaluated as a potential therapy for PD.

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Poster

413. Disease-Modifying Therapy for Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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NIH/NINDS grant R37NS067525

Title: The Role of PAN nuclease in Parkinson's disease

Authors: *H. PARK[1], T.-I. KAM[1], T. M. DAWSON[2], V. L. DAWSON[2];
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Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta and the accumulation and aggregation α-synuclein (α-syn). Misfolded fibrillar forms of α-syn are thought to spread via cell-to-cell communication contributing to the progression and neurodegeneration in PD. However, the underlying molecular mechanisms by which α-syn causes cell death and neurodegeneration in PD is not known. Here, we show that the PAN nuclease mediates α-syn-induced degeneration in A53T transgenic mice. Conditional expression of the familial associated A53T α-syn mutant in DA neurons of transgenic mice leads to loss of DA neurons and behavioral deficits that is significantly reduced in the absence of the PAN nuclease. Further, we developed an endonuclease assay and screened a novel hybrid macrocyclic rapafucin library (~11,248 compoinds) to find PAN nuclease inhibitors. Novel PAN nuclease antagonists can inhibit DNA cleavage and prevent α-syn induced degeneration. Thus, targeting PAN nuclease activity may provide an important therapeutic opportunity in Parkinson's disease.

Disclosures: H. Park: None. **T. Kam:** None. **T.M. Dawson:** None. **V.L. Dawson:** None.

Poster

413. Disease-Modifying Therapy for Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 413.21/S6

Topic: C.03. Parkinson's Disease

Title: The Michael J. Fox Foundation's strategy to generate, characterize, and distribute preclinical alpha-synuclein research tools for molecular biology

Authors: *T. N. MARTINEZ[1], P. H. JENSEN[2], K. C. LUK[3], L. GOTTLER[4], M.-Y. CHOU[5], B. MILLE-BAKER[6], F. VERKAAR[6], C. HABER[7], L. STEINBRUCK[8], H. A. LASHUEL[9], B. FAUVET[9], X. TONG[10], A. L. MORRIS[1], K. D. DAVE[1];
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Abstract: Mutations in the gene SNCA that encodes the protein alpha-synuclein as well as postmortem pathological studies strongly implicate a general role for alpha-synuclein in Parkinson's disease (PD) pathogenesis. Thus, SNCA and alpha-synuclein are attractive targets for developing novel therapeutic approaches for PD patients. A field-wide challenge in PD research however, is a general lack of availability for high-quality, reproducible, and readily accessible preclinical research tools. To address these challenges, The Michael J. Fox Foundation for Parkinson's Research (MJFF) has developed a growing resource of preclinical tools for the PD research and drug development communities that endeavors to provide researchers with easy access to rigorously validated, research-enabling preclinical tools for molecular biology studies. Here we summarize our characterization and validation data for our anti-alpha-synuclein monoclonal antibody which was recently launched for commercial use. This monoclonal antibody is conformation specific for aggregate alpha-synuclein and binds with very high affinity and exquisite specificity for aggregate over monomeric versions of the protein. Both linear and cyclic epitope mapping data for this antibody are also provided. In addition, we describe our recently developed and commercially launched alpha-synuclein protein library, which contains multiple versions of recombinant alpha-synuclein proteins and a pS129 phospho-mimetic peptide. Moreover, we introduce new alpha-synuclein molecular biology reagents that are currently in development within our preclinical tools pipeline, including an alpha-synuclein toxicity and aggregation assay. Ultimately, these MJFF-sponsored alpha-synuclein research tools aim to address field-wide challenges in the preclinical tools and reagents space and overall accelerate PD research.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

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Topic: E.03. Basal Ganglia

Support: MJFF

FRQS

CIHR

NIH R01 NS096240-01

Title: Aldh1a1 is expressed by two dopamine neuron subtypes with distinct molecular and neuroanatomical properties

Authors: *J.-F. POULIN[1], C. M. ESTEP[2], Q. CUI[2], B. HELM[3], C. S. CHAN[2], D. J. SURMEIER[2], R. AWATRAMANI[3];
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Abstract: We recently assessed the molecular diversity of midbrain dopamine neurons, using a single-cell high-throughput gene expression platform based on microfluidic qRT-PCR arrays. This approach revealed multiple molecularly distinct dopamine neuron subtypes, two of which displayed high expression of *Aldh1a1*, a gene belonging to the aldehyde dehydrogenase family. These two dopamine neuron subtypes can be differentiated by the expression of the transcription factors *Sox6* and *Otx2*. To further characterize these two subtypes, we generated a mouse in which the *Aldh1a1* genomic locus was modified to drive the expression of tamoxifen-inducible recombinase CreER^{T2}. We report that one of these subtype is located in the ventral tier of the substantia nigra (SNc) and sends strong projections to the lateral region of the dorsal striatum. The neurons of this subtype were previously found to be more vulnerable in the MPTP model of Parkinson's disease. The other *Aldh1a1*+ subtype is located in the ventral tegmental area (VTA) and projects principally to the medial part of the nucleus accumbens. Electrophysiological analysis that *Aldh1a1*-expressing dopamine neurons in the SNc displayed larger SK and HCN

currents compared to those in the VTA. We are currently exploring the functions of these two dopamine neuron subtypes using the Aldh1a1::CreER^{T2} mouse.

Disclosures: **J. Poulin:** None. **C.M. Estep:** None. **Q. Cui:** None. **B. Helm:** None. **C.S. Chan:** None. **D.J. Surmeier:** None. **R. Awatramani:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Pittsburgh Claude D. Pepper OAIC

William N. & Bernice E. Bumpus Foundation Innovation Award

NIH Grant R01ES020718

Title: Base excision repair variants and pesticide exposure increase Parkinson's disease risk

Authors: *L. H. SANDERS[1], K. C. PAUL[2], E. H. HOWLETT[4], X. HU[4], J. M. BRONSTEIN[3], B. RITZ[2], J. GREENAMYRE[4];
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Abstract: We recently reported selective mitochondrial DNA (mtDNA) damage in the form of abasic sites in the vulnerable nigral neurons in Parkinson's disease (PD). The persistence of abasic sites suggests an ineffective base excision repair (BER) response in PD. In addition, we recently showed that pesticide exposure, which has been linked to PD risk, can cause mtDNA damage. The objective of this study was to investigate the joint impact of variations in genes involved in the BER system and pesticide exposure. To determine whether either *APEX1* rs1130409 or *OGG1* rs1052133 polymorphisms was independently associated with PD, 619 PD patients early in disease and 854 population controls were examined. PD risk was not influenced by either genotype alone. We next investigated whether there were interactions for either BER polymorphism and pesticides. We detected statistically significant (p=0.05) or near significant (p=0.07) interactions for both *APEX1* rs1130409 and *OGG1* rs1052133 and ambient oxidative

stress exposure. At both loci, for pesticide exposed risk allele carriers we estimated more than multiplicative risk increase. Specifically, for *OGG1* rs1052133, the risk was increased by almost 80% in exposed variant carriers (OR=1.79, 95% CI=1.22-2.64). Similarly, for *APEX1* rs1130409, in exposed T allele carriers experienced a 67% increase in risk (OR=1.67, 95% CI=1.13-2.47). The strongest interactions we identified were for the combined BER genetic risk score and pesticide exposures; we detected more than a multiplicative risk with both oxidative stress inducing pesticide exposure (p=0.01) and mitochondrial inhibiting pesticide exposure (p=0.07). The highest risk was estimated for the joint effect of pesticide exposure and the two risk genotypes (mitochondrial inhibitor exposure: OR=2.32, 95% CI=1.44-3.75; oxidative stressor exposure: OR=2.21, 95% CI=1.45-3.38). One of the pesticides analyzed in our study, paraquat, induces oxidative stress by redox cycling. We found mtDNA damage was increased and mitochondrial function impaired following acute paraquat exposure in *in vitro* neuronal cultures. This is the first study to discover variants in BER enzymes interact with pesticide exposure to increase the risk of PD.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant ES020327

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Title: Multifunctional neuroprotection by astrocyte-specific DJ-1 expression in the rotenone model of Parkinson’s disease

Authors: *B. R. DE MIRANDA, E. A. BURTON, J. T. GREENAMYRE;
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Abstract: The Parkinson’s disease (PD) protein DJ-1 accumulates in astrocytes of the substantia nigra (SN) and striatum (ST) of human postmortem tissue from PD patients, indicating a possible compensatory function for this redox sensitive protein in astrocytes. In addition, DJ-1 may be directly involved in the detoxification of alpha-synuclein, the major component of Lewy bodies. DJ-1 has also been reported in humans to act as a transcriptional co-activator of tyrosine

hydroxylase (TH), the enzyme responsible for dopamine production. Further, DJ-1 was reported to upregulate the transcription of the orphan nuclear receptor Nurr1, a protein involved in both the production of TH in developing dopamine neurons, as well as inflammatory gene suppression in mature astrocytes. In the current study, adult male Lewis rats were injected with MuLV-hDJ-1 (or MuLV-GFP control) in the ST and SN, followed by daily rotenone injection (2.8 mg/kg, IP) until motor endpoint was reached. Overexpression of hDJ-1 was associated with profound protection of SN cell bodies ($P<0.001$) and their terminals in ST ($p<0.0001$). To investigate potential mechanisms, histological analyses revealed that animals receiving the MuLV-GFP (control) transgene expressed significantly increased levels of total alpha-synuclein as well as phosphorylated alpha-synuclein (S129; $p<0.01$) within the dopaminergic neurons of the SN following rotenone treatment, a response that was markedly reduced in animals treated with the MuLV-hDJ-1 vector. In addition, the mitochondrial complex I protein NDUFS3 was significantly preserved in dopamine neurons of animals expressing the hDJ-1 transgene in astrocytes, compared to their MuLV-GFP counterparts ($p<0.05$). Protein levels of TH were increased at baseline in the ST of MuLV-hDJ-1 animals ($p<0.01$) and RNA Scope revealed this was a transcriptional effect. Interestingly, Nurr1 expression did not appear to be increased at baseline, but was significantly increased in the dopamine neurons of MuLV-hDJ-1 animals following rotenone treatment, indicating that oxidative stress likely plays a role in the astrocyte-specific modulation of DJ-1 and its downstream functions. In summary, our results indicate that astrocytic overexpression of hDJ-1 is protective in an in vivo model of PD and multiple mechanisms may contribute to this effect.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Program#/Poster#: 414.04/S10

Topic: C.03. Parkinson’s Disease

Title: A longitudinal study of PINK1 and PARK7 KO rats

Authors: *N. W. MILGRAM[1,2], J. A. CASKANETTE[3], A. VAN NIEKERK[3], A. PATRICK[3], L. B. SILENIEKS[4], W. LAU[4], S. THEVARKUNNEL[4], J. A. ARAUJO[4], G. A. HIGGINS[4,2]; [1]Cancog Technologies, Toronto, ON, Canada; [2]U. Toronto, Toronto, ON, Canada; [3]Vivocore, Toronto, ON, Canada; [4]Intervivo Solutions Inc, Toronto, ON, Canada

Abstract: Epidemiological evidence suggests that exposure to environmental toxicants, such as rotenone are a risk factor for Parkinsons Disease (PD). Rotenone is an industrial pesticide that

induces toxicity by affecting complex I mitochondrial function and consequent oxidative damage. Various PD associated genes have also been identified, including Park7 (DJ-1) and Pink1, both of which have a role in mitochondrial homeostasis. Accordingly, both Park7 KO and Pink1 KO rats have been created using Zinc Finger Nuclease (ZFN) technology. A recent report (Dave et al (2014) Neurobiol. Dis. 70: 190-203) described motor changes emerging at 6-8 months age, notably in the Pink1 KO, and evidence for loss of DAergic neurons within the substantia nigra of both KO lines at 8 months. The purpose of the present study was to examine the motor behavioural phenotype of both the Pink1 and Park7 KO lines over a longer timespan (4-20 months age), and to examine the interaction of low dose rotenone administered chronically (3 x 0.5 mg/kg IP/week) on this phenotype. A total of 24 male Park7 KO and 24 Pink1 KO rats entered the study, with 24 of their respective wild type controls, i.e 96 rats in total. Twelve rats from each group received a saline injection 3x weekly, while the other 12 rats received a rotenone injection 3x weekly. Each week from W10 to W88, rats were scored on a simple 5 point scale based on hindlimb and general motor function (0=normal, 4=hindlimb paralysis). Bimonthly neurological assessments were also made, including measures of locomotor activity, beam walking, postural instability. At 20 months (W88) measures of cognitive function (novel object recognition task) and motor response to amphetamine challenge were also taken. The Park7 KO rats developed a modest neurological phenotype over the study duration. At W10 all scores were 0, and by W88, the Park7 KO group was significantly higher than wild type controls with a modest interaction with rotenone treatment (WT+veh: 0$\pm$0; WT+ROT: 0$\pm$0; Park7+VEH: 1.5$\pm$0.3; Park7+ROT: 2.5$\pm$0.4). The Pink1 KO line showed a distinct profile. At W26-32 the Pink1 KO rats had developed a robust neurological phenotype characterized by hindlimb weakness and dragging (e.g. W28 Pink1+VEH: 2.8$\pm$0.2), however by W52 these scores had declined to near WT levels (W52 Pink1+VEH: 0.9$\pm$0.2) suggesting a return of normal hindlimb function. This recovery was also confirmed by other indices of motor function. By W88 neurological scores had increased slightly but were still lower than W26-32. These studies thus characterize the motor phenotype of both KO lines over an extended lifespan and may suggest a subtle interaction between Park7 gene KO and chronic low dose rotenone exposure.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS067024

Title: Rab8A effects on alpha-synuclein toxicity in a rat model of Parkinsonism

Authors: *N. R. MCFARLAND[1], H.-J. PARK[2], D. RYU[2], L. POWELL[2], R. FOELS[2], M. PARMAR[2];
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Abstract: A major pathological hallmark of Parkinson disease is the presence of Lewy bodies that are enriched with the protein, alpha-synuclein (αS). Abnormal accumulation and deposition of αS are associated with ER stress, intracellular vesicle trafficking deficits, and cytotoxicity. Rab GTPases play critical roles in intracellular protein and vesicle trafficking, and expression of specific Rabs has been shown to ameliorate trafficking deficits and toxicity caused by αS overexpression. These findings suggest not only that abnormal accumulation of αS may interfere with normal Rab protein functions, but that RabGTPases also may play important role in αS homeostasis and reduce formation of toxic oligomers or aggregate formation. We and others have shown that Rab8A reduces αSyn and associated oligomer and aggregate formation in cellular models. In this study we aimed to test the impact of viral-mediated Rab8A expression on αSyn pathology *in vivo* in an adult rat model of Parkinsonism. We hypothesized that targeted viral expression of Rab8A in the substantia nigra would reduce formation of "toxic" αS oligomeric species, aggregates, and nigrostriatal dopamine toxicity. For this study adult Sprague dawley rats were unilaterally injected stereotaxically in the substantia nigra (SN) with recombinant adeno-associated virus (rAAV) serotype 2/8, expressing human αS (AAV-CBA-Syn-WPRE construct) or empty vector plus or minus N-terminal Flag-tagged Rab8A in 2 µL volume. All virus were individually tested at several titers for effects and nigrostriatal toxicity, and the concentration for AAV-Rab8A specifically chosen due to lack of independent toxicity in preliminary tests. At 8 weeks post injection, rats were sacrificed and brains extracted for analyses. The results demonstrate robust co-expression αS and Rab8A in animals that received combined rAAV injection. Viral expression of Rab8A alone (with empty vector) resulted in greater than expected dopamine cell loss (38.3%) in the SN as measured by tyrosine hydroxylase (TH) immunostaining. As expected αS expression caused significant TH cell loss at 8 weeks (65.4%), but when co-expressed with Rab8A there was no significant rescue (58.9%) of αS-induced TH cell loss in the SN. Similarly, there was no difference in relative TH expression in striatal tissues from rats injected with rAAV expressing Rab8A compared to those with empty vector. Together, these preliminary findings indicate that viral expression Rab8A is not sufficient to protect against αS toxicity in the rat nigrostriatal system. However, further studies are needed to assess adequate expression and effects on αS levels, aggregate formation, and toxicity.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Increase of tau protein levels in sporadic and experimental parkinson's disease

Authors: *Y. CHU[1], Y. HE[2], J. H. KORDOWER[2];
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Abstract: Alpha-synuclein and tau are abundant brain proteins with distinct biological functions and intraneuronal distribution. Alpha-synuclein is mainly localized in axon terminals where it may regulate synaptic functions whereas tau is a microtubule binding protein that stabilizes and promotes microtubule polymerization. However, alpha-synuclein and tau are both partially unfolded proteins that can form toxic oligomers and abnormal intracellular aggregates under pathological conditions. Alpha-synuclein positive inclusions are also described in tauopathies and *vice versa*, suggesting a co-existence or crosstalk between these proteinopathies. The purpose of our study was to examine whether abnormal alpha-synuclein can induce tau accumulation in Parkinson's disease (PD). Twelve human brains from PD (n=6) and age-matched controls (n=6) as well as twelve rat brains receiving AAV-alpha-synuclein (n=6) or empty vector (n=6) were analyzed using immunohistochemistry. The colocalization of alpha-synuclein and tau accumulation was examined with immunofluorescent double labeling. The levels of tau were determined using densitometry. Colocalization analyses revealed that accumulated tau was observed in both nigral neurons with or without alpha-synuclein inclusions in PD. Compared to age-matched controls, a marked increase in perikarya tau immunoreactivities was observed in substantia nigra of PD. Increase in tau levels were recapitulated in a rat model of PD based on viral over-expression of human wild-type α-synuclein, suggesting that α-synuclein overexpression appear to promote tau accumulation in perikarya of nigral neurons. Our preliminary data demonstrate that interactions between tau and α-synuclein may form a deleterious feed-forward loop essential for the development and spreading of neurodegeneration.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: National Foundation of China No.20435020

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Title: The effect of N-methylsasolinol on α-synuclein transgenic PD model

Authors: Z. CHEN, *H. MA, F. SUN, Y. DENG;
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Abstract: Parkinson’s disease(PD) is the second most common neurodegenerative disorder. The number of PD patients is expected to reach 10 million in the next 20 years worldwide,which will pose a huge burden on society.Loss of dopaminergic neuron in substantia nigra pars compacta (SNpc) and protein aggregation named lewy Bodies(LBs) are two important hallmarks of PD.A growing body of evidences suggested that CAIQ can cause the death of dopaminergic neurons including N-methylsasolinol(NMSal),which has been considered as one kind of endogenous neurotoxins to induce Parkinson's disease(PD).Aggregated α-synuclein(α-syn) is a major component of lewy bodies and the pathological changes of α-syn are considered to implicate in PD.In our project,we investigated whether CAIQ can enhance α-syn aggregation,lead to dopaminergic neurodegeneration and try to clarify the exact molecular mechanism of PD.We established α-syn transgenic rat model using adult male wistar rats through injecting α-syn overexpression recombinant adeno-associated viral (rAAV) by cerebral stereotaxic technique to the right SNpc of rats.After 12 weeks, the time node behavior test was performed and the rats showed apomorphine induced rotation to the left side which is a characteristic behavior to loss of dopaminergic neurons. The effect of NMSal on dopamine and its metabolites, oxidative stress,mitochondrial damage and α-syn aggregation will be evaluated. 100nmol NMSal was injected to the same site with rAAV injection and the behavior test showed that the NMSal accelerated apomorphine induced rotation in rAAV-α-synuclein groups after 4 weeks post injection.The levels of malondialdehyde(MDA),reactive oxygen species(ROS),endogenous neurotoxins(Salsolinol and NMSal)and loss of dopaminergic neurons were all elevated in the α-syn transgenic rats after NMSal induction. In addition,we also observed that NMSal enhanced the aggregation of α-syn.These results indicated that NMSal can enhance the dopaminergic neurotoxin induced by α-syn overexpression. Moreover,we further explored the effect mechanism of NMSal on α-syn aggregation by detecting the degradation pathways of α-syn.The results showed that NMSal can inhibit the expression of Beclin-1, Atg5 and Atg3 which are associated with autophagy-lysosomal pathway(ALP).So NMSal may increase α-syn aggregation

by inhibiting the degradation of α-syn through autophagy-lysosomal pathway(ALP). Our project provided a evidence that the interaction between NMSal and α-syn might be involved in PD pathogenesis.This study will give a new insight into the development and treatment of PD.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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University of Bordeaux Visiting Scholar Program

MARC program

Title: The effect of zinc administration in Atp13a2-deficient mice

Authors: *N. A. SANTIAGO[1], R. BLACKWOOD[2], S. HUBBARD[2], S. S. KARKARE[2], E. R. DIRR[2], G. E. SHULL[3], E. MASLIAH[4], B. LIOU[5], Y. SUN[5], P.-O. FERNAGUT[6], E. BEZARD[6], B. DEHAY[6], S. M. FLEMING[1];
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Abstract: Loss of function mutations in the gene *ATP13A2* are associated with the neurodegenerative diseases Kufor-Rakeb Syndrome and Neuronal Ceroid Lipofuscinosis. The function of ATP13A2 is unclear but in vitro studies suggest that it is involved in the lysosomal degradation of proteins and in the homeostasis of heavy metals including zinc and manganese. We previously showed that *Atp13a2-deficient* (13a2) mice develop age-dependent sensorimotor deficits, enhanced lipofuscinosis, and accumulation of insoluble alpha-synuclein (aSyn) in the brain. More recently, we also showed they are more sensitive to the toxic effects of manganese administration. These findings suggest ATP13A2 may be a key protein involved in gene-environment interactions that contribute to neurodegenerative conditions. In the present study, two cohorts of mice were treated with a zinc chloride solution. In the first cohort, two-four month old wildtype (WT) and 13a2 mice received four subcutaneous injections over the course of 14 days of 20 mg/kg zinc chloride or vehicle. Soluble and insoluble aSyn levels were then

determined in the dorsal and ventral midbrain. In the second cohort WT, 13a2, aSyn overexpressing (aSyn), and double mutant 13a2-aSyn mice were administered a low dose of zinc chloride solution (1 mg/kg) by oral gavage chronically twice a week for four months beginning at two months of age. At six months of age, cathepsin D, p62, and LAMP1 expression levels were measured in the striatum, ventral midbrain, and cortex. Results from the first cohort show that soluble aSyn levels were significantly increased in the dorsal midbrain but not ventral midbrain in 13a2 mice receiving zinc compared to vehicle-treated WT. No changes in insoluble aSyn were detected in either region. In the second cohort, zinc-treated double mutant 13a2-aSyn mice had significantly increased levels of LAMP1 and p62 and significantly decreased levels of cathepsin D in the striatum compared to zinc-treated WT mice. These data indicate loss of ATP13A2 function in vivo leads to increased sensitivity to zinc and region specific alterations in aSyn accumulation and lysosomal function.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Title: Nuclear translocation of SIRT2 induced by Cdk5 dependent phosphorylation promotes the dopaminergic neuron death in Parkinson's disease

Authors: ***J. YAN**, P. ZHANG, F. HE, F. JIAO, Q. WANG, L. CHEN, Q. ZHANG, B. TIAN; Dept. of Neurobiology,Tongji Med. Sch., Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: Parkinson’s disease (PD), commonly known as a secondary degenerative disease of the central nervous system (CNS),is characterized with the progressive loss of dopaminergic (DA) neurons within the SNpc (substantia nigra pars compacta). NAD-dependent protein deacetylases Sirtuin 2 (SIRT2), which mediates multiple cellular pathways by deacetylating its various substrates, has been extensively investigated in Parkinson’s disease. Although numerous

publications were shown SIRT2 deletion has beneficial effects against dopaminergic neuron loss in the 1-methyl-4-phenyl-1, 2, 5, 6-tetrahydropyrine (MPTP) model of Parkinson's disease, the precisely mechanisms under SIRT2 mediates neuronal death have remained largely unknown. Here, we show that ablating of SIRT2 could rescue PD, TH positive neurons within SNpc and behavior test were performed in SIRT2 KO (knockout) mice after systemic administration of MPTP via the intraperitoneal route and the α-synuclein-A30P*A53T double mutant transgenic mice of PD lacking of SIRT2. The mRNA and total protein level of SIRT2 were not altered in animal and cell model of PD detected by RT-PCR and Western Blot. Importantly, SIRT2 translocate from cytoplasm to nucleus in Parkinson's disease cell model and animal model, as well as the nuclear translocation SIRT2 could promotes neuronal death. Furthermore, Cdk5 (Cyclin-Dependent Kinase 5) was required for the translocation of SIRT2 by the Ser331 and Ser335 sites phosphorylation. On the basis of our data, a short peptide named Myr-SIRT2$_{328-339}$, targeting competitive inhibition of Cdk5-dependent Ser331 and Ser335 phosphorylation within SIRT2, was designed and conjugated myristic to ensure its cell-penetrating ability. Unsurprisingly, Myr-SIRT2$_{328-339}$ could obviously decrease the mortality of primary culture neurons on the condition that MPP^+ treated. In conclusion,these results implicate a novel mechanism of regulating neuronal death by Cdk5-dependent nuclear-cytoplasmic shuttling of SIRT2 in the progression of Parkinson's disease. Future studies aimed at further elucidating the exact mechanistic action of the nuclear localization of SIRT2 are essential for promoting the dopaminergic neuronal death, thus providing additionally therapeutic target in PD.

Disclosures: J. Yan: None. **P. Zhang:** None. **F. He:** None. **F. Jiao:** None. **Q. Wang:** None. **L. Chen:** None. **Q. Zhang:** None. **B. Tian:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.10/T2

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation for Parkinson's Research

Title: Dysregulation of protein phosphatase 2A in dementia with lewy bodies

Authors: H.-J. PARK[1], K.-W. LEE[1], E. PARK[1], S. OH[1], R. YAN[1], J. ZHANG[1], T. BEACH[2], C. ADLER[3], M. VORONKOV[4], S. BRAITHWAITE[4], J. STOCK[4,5], *M. M. MOURADIAN[1]; [1]Neurol., Rutgers-Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Banner Sun Hlth. Res. Inst., Sun City, AZ; [3]Mayo Clin., Scottsdale, AZ; [4]Signum Biosci., Princeton, NJ; [5]Mol. Biol., Princeton Univ., Princeton, NJ

Abstract: Protein phosphatase 2A (PP2A) functions as a master regulator of cellular phosphoregulatory networks in the brain, controlling key processes required for protein homeostasis and cell survival. The heterotrimeric holoenzyme is composed of a highly conserved catalytic C subunit, a scaffold-like A subunit, and one of several regulatory B subunits that confer substrate specificity. The assembly and activity of PP2A is regulated by reversible carboxyl methylation of the C subunit. α-Synuclein, which misfolds and aggregates in hallmark pathologic lesions of Dementia with Lewy Bodies (DLB), is phosphorylated at Ser_{129}, and PP2A containing a B55α subunit is a major phospho-Ser_{129} phosphatase. To investigate the possibility that PP2A dysregulation could play a role in the pathogenesis of DLB, we have compared the state of PP2A methylation, as well as the expression of its methylating enzyme, leucine carboxyl methyltransferase (LCMT-1), and demethylating enzyme, protein phosphatase methylesterase (PME-1), in postmortem brain tissues from 8 DLB cases and an equal number of age-matched non-neurological controls. Mean age at death was 82 ± 2.6 years for DLB and 84 ± 2.1 years for Controls. Postmortem interval was ≤ 3 hours in all subjects. Mean Mini Mental State Examination scores were 8.8 ± 3.1 in DLB and 27.1 ± 0.8 in Controls ($p < 0.01$). Neuropathological stage of α-synucleinopathy assigned according to the Unified Staging System for Lewy Body Disorders showed all DLB cases to be in stage 4, and all the Controls were in stage zero. Concomitant Alzheimer pathology was also present. Immunohistochemical studies showed that LCMT-1 was significantly reduced in both frontal cortex and substantia nigra of DLB cases compared to Controls, while PME-1 was not significantly altered. This was associated with a reduction in the ratio of methylated PP2A to demethylated PP2A in DLB. These findings support the hypothesis that PP2A dysregulation in α-synucleinopathies may contribute to the accumulation of hyperphosphorylated α-synuclein and to the disease process, raising the possibility that pharmacological means to enhance PP2A phosphatase activity may be a useful disease modifying therapeutic approach.

Disclosures: **H. Park:** None. **K. Lee:** None. **E. Park:** None. **S. Oh:** None. **R. Yan:** None. **J. Zhang:** None. **T. Beach:** None. **C. Adler:** None. **M. Voronkov:** None. **S. Braithwaite:** None. **J. Stock:** None. **M.M. Mouradian:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.11/T3

Topic: C.03. Parkinson’s Disease

Title: characterization of n370s gba1-PD ips cells derived human dopaminergic neurons

Authors: *S. YUN, H. KO;
Neurol., Johns Hopkin Med., Lutherville Timonium, MD

Abstract: Mutations in glucocerebrosidase1 (GBA1) are the most common genetic risk factor for development of Parkinson's disease. Here we generate several iPSC cell lines from sporadic PD patients with or without N370S GBA1 mutation, the most frequent mutation. Employing a floor-plate-based strategy, we differentiate the iPSC cell lines into human dopaminergic (DA) neurons. Human dopaminergic neurons derived from N370S GBA1-PD iPSCs exhibit several biochemical and cellular defects including decreased GBA1 protein levels and enzyme activity and subsequent accumulation of α-synuclein, mitochondrial defects, reduced lysosomal β-galactosidase enzymes activity, as well as susceptibility to α-synuclein preformed fibrils (PFFs) induced LB/LB-like pathology and human DA neuron death. Moreover, the levels of total glucosylceramide and glucosylsphingosine, substrates of GBA1, are increased in N370S GBA1-PD iPSCs derived human DA neurons as assessed by quantitative lipidomic analysis. Strikingly, overexpression of wild type GBA1 reverses such biochemical and cellular defects observed in N370S GBA1-PD iPSCs derived human neurons. We are currently conducting RNAseq to identify changes in transcriptome due to N370S GBA1 mutation. Taken together, the N370S GBA1-PD iPSCs recapitulate biochemical and cellular phenotypes of GBA associated PD and provide unlimited, stable and genetically tractable sources for understating molecular mechanisms of PD due to GBA mutation and a drug-screening platform for preclinical drug discovery for the GBA-associated PD therapy.

Disclosures: S. Yun: None. **H. Ko:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.12/T4

Topic: C.03. Parkinson's Disease

Title: Alterations in ganglioside biosynthesis and content in Parkinson's disease and role in modulating vulnerability for neurodegeneration

Authors: *M. VERMA[1], S. JACKSON[2], T. N. SEYFRIED[3], H.-S. CHOI[3], Z. AKGOC[3], A. LYNN[3], J. S. SCHNEIDER[1];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Thermo Fisher Scientific, South San Francisco, CA; [3]Boston Col., Chestnut Hill, MA

Abstract: We have previously shown that administration of GM1 ganglioside to Parkinson's disease (PD) patients results in a lower than expected rate of symptom progression. Decreased expression of GM1 in PD substantia nigra (SN) could increase the vulnerability of dopamine (DA) neurons to damage and administration of GM1 may have restored a sufficient amount of GM1 to enhance functionality and stabilize/protect these neurons. Alterations in ganglioside biosynthesis and expression have been observed in association with aging and may occur in some neurodegenerative diseases (such as Huntington's disease). The extent to which such changes occur in the PD brain are unclear as is the potential role that such changes might play in enhancing the vulnerability of DA neurons to neurodegeneration in response to various stressors. The expression of various glycosyltranferases and sialyltransfereases involved in ganglioside biosynthesis (as well as other genes representing various biochemical pathways associated with glycolipid synthesis and metabolism and select additional genes of potential relevance to PD and neuroprotection) was examined by laser capture microdissection of substantia nigra (SN) DA neurons followed by RNA extraction, generation of RNA amplicon libraries for use with a custom Ampliseq glycomics panel and Next-Gen sequencing using the Ion PGM System. Numerous genes were highly down-regulated in PD DA neurons (compared to age/sex-matched normal control DA neurons) and included *B3Galt4, St8Sia5, St3Gal2, FGF2, FGFR1*. qPCR also showed decreased *B3Galt4* and *St3Gal2* expression in PD SN extracts. Analysis of total gangliosides and ganglioside distribution in PD and control SN showed total ganglioside content, GD1a, GD1b, and GT1b to be significantly decreased in PD SN compared to control. GM1 was also decreased but this did not reach statistical significance. We then examined the extent to which a decrease in *B3Galt4* expression might enhance vulnerability to a low level exposure of a potential toxicant. Exposure of differentiated SK-N-SH DAergic cells to *B3galt4* siRNA significantly decreased GM1 expression and resulted in significant cell death in response to an exposure (10μM MPP^+) that previously resulted in no cell loss. These results suggest that the glycolipid defect in SN DA neurons in PD may be more extensive than previously thought and that changes to the integrity of glycolipids in the PD brain may play an important role in the pathogenesis of PD.

Disclosures: **M. Verma:** None. **S. Jackson:** None. **T.N. Seyfried:** None. **H. Choi:** None. **Z. Akgoc:** None. **A. Lynn:** None. **J.S. Schneider:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.13/T5

Topic: C.03. Parkinson's Disease

Support: Fonds National de la Recherche Luxembourg

Title: Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3d microfluidic cell culture

Authors: *E. LUCUMI MORENO[1], S. HACHI[1], K. HEMMER[1], S. J. TRIETSCH[2], A. S. BAUMURATOV[1], T. HANKEMEIER[3], P. VULTO[2], J. C. SCHWAMBORN[1], R. M. T. FLEMING[1];
[1]Univ. of Luxembourg, Campus Belval, Belvaux, Luxembourg; [2]Mimetas, Leiden, Netherlands; [3]Leiden Univ., Leiden, Netherlands

Abstract: A hallmark of Parkinson's disease is the progressive loss of nigrostriatal dopaminergic neurons. We derived human neuroepithelial cells from induced pluripotent stem cells and successfully differentiated them into dopaminergic neurons within phase-guided, three-dimensional microfluidic cell culture bioreactors. After 30 days of differentiation within the microfluidic bioreactors, in situ morphological, immunocytochemical and calcium imaging confirmed the presence of dopaminergic neurons that were spontaneously electrophysiologically active, a characteristic feature of nigrostriatal dopaminergic neurons in vivo. Differentiation was as efficient as in macroscopic culture, with up to 19% of differentiated neurons immunoreactive for tyrosine hydroxylase, the penultimate enzyme in the synthesis of dopamine. This new microfluidic cell culture model integrates the latest innovations in developmental biology and microfluidic cell culture to generate a biologically realistic and economically efficient route to personalised drug discovery for Parkinson's disease.

Disclosures: **E. Lucumi Moreno:** None. **S. Hachi:** None. **K. Hemmer:** None. **S.J. Trietsch:** None. **A.S. Baumuratov:** None. **T. Hankemeier:** None. **P. Vulto:** None. **J.C. Schwamborn:** None. **R.M.T. Fleming:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Program#/Poster#: 414.14/T6

Topic: C.03. Parkinson’s Disease

Support: MOST 1032320B006005MY2

Title: Increased Kv2.1 channel expression contributes to nigrostriatal degeneration in MPTP induced Parkinson’s disease model

Authors: R.-Y. CHAO[1], C.-H. CHENG[1], *P.-C. CHEN[2];
[1]Physiol., Natinal Cheng Kung Univ., Tainan, Taiwan; [2]Physiol., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Parkinson's disease is due to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc DA neuron) and the degeneration of dopaminergic axon terminals the dorsal stratum. Kv2.1, a voltage gated potassium channel enhanced K^+ efflux has been shown to be an essential mediator in programmed cell death and oxidative aggregation of Kv2.1 may be a key event in both healthy aging and neurodegenerative diseases. Although Kv2.1 channel has not been reported in the regulation of pacemaker activity in SNpc dopamine neurons, Kv2.1 channel expression can be detected in DA neurons across postnatal development. First, we asked whether Kv2.1 channel is involved in the nigrostriatal degeneration using 1-metyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's disease mouse model. Mice were injected with 30 mg/kg of MPTP for 5 days, withdrawal for 7 days and sacrificed at day 7. We observed the loss of DA neurons in SNpc and DA axon terminals in the striatum. Interestingly, there was an increase of Kv2.1 channel expression in the SNpc and striatum after MPTP injection. Quantitative PCR analysis revealed that no changes in mRNA level between the vehicle and MPTP groups. Then, for the distribution of Kv2.1 channels in the striatum or SNpc, vehicle treated mice had more punctate staining of Kv2.1 channel in both the SNpc and stratum than MPTP treated mice. To characterize what type of neuron express Kv2.1 channels, we co-stained Kv2.1 and DARPP-32 which is the marker for striatal medium spiny neurons. Co-staining results indicated Kv2.1 channel is located in medium spiny neurons. Next, to understand whether this increase of Kv2.1 channel is necessary for MPTP-induced nigorstriatal degeneration, mice pre-treated with Kv2.1 blocker (0.11mg/kg, Guangxitoxin 1E, GxTx) followed by MPTP injection showed more DA neurons in the SNpc and DA axon terminal in the striatum using immunoblotting and immunostaining assays, suggesting that inhibition of Kv2.1 channel protects DA neurons from MPTP toxicity. In parallel, MPP^+ dose dependently increased the expression of Kv2.1 channel in dopaminergic cell lines (MN9D, SY5Y) and primary cortical neuron culture. We confirmed the results by caspase 3 immunostaining and cell viability assay. Also, pre-treatment with GxTx inhibited MPP^+ induced Kv2.1 channel increase in MN9D cells. In conclusion, our results suggest that increased expression of Kv2.1 channel contributes to nigrostriatal degeneration. In the future, we will primarily focus on the investigation of underlying mechanism by which MPTP regulated the increase of Kv2.1 channel expression and continue to improve the quality for the data.

Disclosures: R. Chao: None. **C. Cheng:** None. **P. Chen:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.15/T7

Topic: C.03. Parkinson's Disease

Support: FAPESP

CNPq (Brazil)

Title: A possible involvement of TRPM7 in the 6-hydroxy-dopamine model of Parkinsons disease

Authors: *L. M. DATI[1], H. ULRICH[1], H.-S. SUN[2], Z.-P. FENG[2], L. BRITTO[1];
[1]Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson´s disease (PD) involves a loss of dopaminergic neurons of the substantia nigra, which produces marked motor and cognitive disorders. PD has been studied with several approaches, including animal models such as the 6-OH-dopamine intrastriatal injection. Since transient receptor potential TRPM7 channels have been recently demonstrated to be involved in some neurodegeneration conditions, we sought to investigate a possible participation of TRPM7 in the pathophysiology of PD-like condition in the mouse, this receptor is binding with G-protein, so is important to regulated the cell. This work used techniques by means of immunohistochemistry and Western blotting. Tyrosine-hydroxylase immunolabeling in wild-type mice (C57BL/6) was reduced by about 50% both in the striatum and the substantia nigra on the side ipsilateral to the 6-OH-dopamine injection in relation to the contralateral side, injected with saline. TRPM7 is increased in both the striatum (ca. 80%, $p<0.005$) and substantia nigra (ca. 50%, $p<0.005$) after injections of 6-OHDA. We have then used a TRPM7 inhibitor, carvacrol (40mg/Kg, i.p.), the animals had 30 minutes after the put carvacrol for admintration of 6-OHDA, and evaluated tyrosine-hydroxylase expression in both control and 6-OH-dopamine-injected animals. The results showed that, in the striatum, the reduction of dopaminergic innervation observed in control animals was markedly attenuated (ca. 72%) in carvacrol-treated mice. The same was observed for the substantia nigra, as the typical reduction of the number of tyrosine-hydroxylase positive neurons after 6-OH-dopamine was not significant in carvacrol-treated mice (attenuation of ca. 85%). This compare is about group carvacrol with 6-OHDA for saline anda 6-OHDA. When compare the group carvacrol with 6-OHDA, it's possible see that in striatun has the increased about 17%, but in substancia nigra is decreased about 13%, this not different the control but is different with group saline.In order to preliminarily investigate the mechanisms of the carvacrol-induced neuroprotection, we analyzed the expression of caspase 3 with the same protocols. There was an increase of caspase 3 expression of about 90% ($p<0.005$) in both the striatum and the substantia nigra after 6-OH-dopamine injections, whereas the carvacrol-treated

mice did not show appreciable increases of caspase 3, so this group stay similar the control. These results suggest that TRPM7 is involved in the pathophysiology of the 6-OH-dopamine model of Parkinson´s disease, possibly by increasing caspase 3 levels.

Disclosures: L.M. Dati: None. **H. Ulrich:** None. **H. Sun:** None. **Z. Feng:** None. **L. Britto:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.16/T8

Topic: C.03. Parkinson's Disease

Support: FAPESP

CAPES

CNPq

UNICID

Title: Neuroplasticity induced by acrobatic exercise in a rat model of Parkinson's disease.

Authors: *R. S. PIRES[1], N. R. SANTIAGO[1], Q. R. S. G. FERRAREZI[1], R. O. JACOB[1], C. C. REAL[2], L. R. G. BRITTO[2];
[1]Univ. Cidade De São Paulo, Sao Paulo, Brazil; [2]Dept. Physiol. and Biophysics, Univ. of São Paulo, São Paulo, Brazil

Abstract: Parkinson's disease (PD) is a neurodegenerative and progressive disease that promotes motor, cognitive and emotional deficits that are ameliorated with treadmill and wheel running exercise. Nevertheless, little is known about the effects of acrobatic exercise, which involves complex motor tasks requiring intense activation of basal nuclei-thalamus-cortical circuit, which is compromised in PD. Therefore, our objective was to analyze neuroprotective effects and plasticity changes in motor regions of the brain of rats submitted to unilateral 6-hydroxydopamine (6-OHDA) PD model and acrobatic exercise protocol. Forty-eight animals were divided into 2 groups, namely Parkinson's (PD) and Control-saline (SAL), and each one was subdivided into acrobatic (AC) and sedentary (SED). The AC moved through a circuit of obstacles 5 times/day, 3 days/week for 4 weeks. One month after starting the training, their brains were removed for immunohistochemistry and Western blotting assays to analyze the expression of microtubule-associated 2 (MAP2), synaptophysin (SYP) and tyrosine hydroxylase

(TH) in the prefrontal cortex, motor cortex, dorsolateral and dorsomedial striatum and substantia nigra pars compacta (SNc). Statistical analyses were performed using one-way ANOVA with the Bonferroni *post hoc* test. PD+SED group showed a decrease in the percentage of TH-positive cells in the SNc (ca. 48%, $p < 0.0001$) when compared to SAL+SED, but the PD+AC group had a higher number of TH+ cells (ca. 40%, $p<0.0001$) compared to SED. In the striatum, 6-OHDA produced a significant TH, MAP2 and SYP decrease in the dorsomedial (ca. 30%, $p<0.0001$; ca. 31%, $p < 0.0001$; ca. 29%; $p<0.0001$; respectively) and dorsolateral striatum in the PD+SED (ca. 29%, $p < 0.0001$; ca. 47 %, $p<0.0001$; ca. 35%, $p<0.0001$; respectively) compared to SAL+SED. However, PD+AC showed a TH, MAP2 and SYP increase in the dorsomedial (ca. 17%, $p<0.0001$; ca. 12%, $p<0.0001$; ca. 11%, $p<0.01$; respectively) and dorsolateral (TH- ca. 17%, $p<0.0001$ and MAP2 - ca. 38%, $p< 0.0001$) areas compared to PD+SED. In the motor cortex and prefrontal cortex, both PD+SED and PD+AC revealed no changes in the MAP2 and SYP expression compared to SAL+SED and SAL+AC. In conclusion, our data suggested that four weeks of AC exercise induced a neural circuit reorganization, mainly in nigrostriatal areas that are injured in PD.

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Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.17/T9

Topic: C.03. Parkinson’s Disease

Title: 6-hydroxydopamine- and L-DOPA-induced shifts in striatal monoamine transporter function in L-DOPA-primed, hemi-parkinsonian rats

Authors: *M. CONTI, S. MEADOWS, J. GOLD, N. PALUMBO, J. HALLMARK, D. WERNER, C. BISHOP;
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Motor symptoms resulting from dopamine (DA) cell loss seen in Parkinson’s disease are typically treated with the DA precursor L-DOPA. However, chronic treatment usually results in abnormal involuntary movements (AIMs) referred to as L-DOPA-induced dyskinesia (LID). Growing evidence suggests that mechanisms underlying LID involve interactions between the remaining DA system and serotonin (5-HT) and norepinephrine (NE) systems. Specifically, 5-HT and NE transporters (SERT and NET, respectively) gain function in L-DOPA-derived DA uptake when DA transporter (DAT) levels are low. Recently, our lab has shown that DAT,

SERT, and NET inhibition with monoamine transporter-specific blockers differentially modulate LID expression, with SERT blockade having the greatest effect. Although a functional shift can be inferred from behavioral measures, a structural shift in striatal DAT, SERT, and NET expression has not been well characterized. Therefore, the current experiment sought to determine how severe DA loss and subsequent L-DOPA treatment affected striatal DAT, SERT, and NET levels in unilateral sham- or 6-OHDA-lesioned Sprague-Dawley rats. After a 3 week recovery period, rats were sorted into equal groups based on motor performance. Rats were then primed with vehicle or L-DOPA (12 mg/kg + benserazide 15 mg/kg; s.c.) and monitored for AIMs expression. After a 1 week washout period, rats were given daily vehicle or L-DOPA (6 mg/kg) for 2 weeks while being monitored for AIMs expression and off treatment motor performance. One hour after rats received their last respective treatment, lesioned striatal tissue was taken for whole cell western blot analysis of total DAT, SERT, and NET expression. Behaviorally, DA-lesioned rats had worse motor performance than sham-lesioned rats and only the L-DOPA treated, DA-lesioned group exhibited significant LID. Lesion-induced DAT loss mirrored motor deficit while SERT and NET expression was unaffected by DA depletion or L-DOPA treatment. Interestingly, SERT:DAT and NET:DAT increased in the L-DOPA treated, DA-lesioned group which significantly correlated with LID severity. Overall, severe DA loss and L-DOPA treatment appear to functionally promote SERT and NET without affecting cellular expression in the dyskinetic striatum.

Disclosures: **M. Conti:** None. **S. Meadows:** None. **J. Gold:** None. **N. Palumbo:** None. **J. Hallmark:** None. **D. Werner:** None. **C. Bishop:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.18/T10

Topic: C.03. Parkinson’s Disease

Support: Division of Life Science and Applied Life Science (BK 21 plus)

Title: Neuroprotective role of Osmotin in alpha-synuclein induced neuropathology via AMPK activated signaling cascade.

Authors: ***M. JO**, M. KHAN, M. SOHAIL, M. KIM;
Neurobio. Lab., Jinju, Korea, Republic of

Abstract: Studies of neurodegenerative diseases have focused on the physiological and pathological abnormalities of specific neuronal cell populations. Abnormal neuronal aggregation

of α-synuclein is implicated in the development of many neurological disorders. Natural products have been a focus of scientific research as therapeutic agent for prevention and treatment of neuro-degenerative disease. Osmotin is a pathogenesis-related (PR) plant protein is a homolog of the mammalian adiponectin hormone. Previously it's been reported that Adiponectin negatively regulate the pathogenesis of α-synucleinopathies in an animal model. In present study an in vitro model over expressing a-synuclein in SH-SY5Y human neuroblastoma cells treated MPP+ to mimic α-synucleiopathy been designed. Osmotin treatment shows diminution in α-synuclein overexpression as compared to untreated samples. AMPK is a master regulator believed to play a critical role in the control of energy homeostasis and survival of neurons. Present study shows that osmotin restores AMPK activity interrupted by enhanced expression of mTOR associated proteins like LKB1 and RPTOR protein responsible for neurotoxicity of α-synuclein in disease model. Osmotin not only reduced α-synuclein expression but also enhances cell survival and synaptic neurotransmission through enhanced dopamine sysnthesis. Present study elaborates therapeutic potential of osmotin against α-synuclein induced neuropathy by activation of AMPK and its associated downstream signaling proteins.

Disclosures: **M. Jo:** None. **M. Khan:** None. **M. Sohail:** None. **M. Kim:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 414.19/T11

Topic: C.03. Parkinson's Disease

Title: Behavioural effects of sodium benzoate, a cinnamon metabolite, on the 6-hydroxydopamine rat model of parkinson's disease.

Authors: ***M. K. SHAMMAS**[1], R. T. KENNEDY[2];
[1]Neurosci., [2]Chem. and Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's. Its main treatment, levodopa (L-DOPA), has side effects, most prominently dyskinesia, that become worse over time, and in some cases become more intolerable than the disease itself. A recent study (J Neuroimmune Pharmacol. 2014; 9:569-81) has shown that sodium benzoate (NaB), a metabolite of cinnamon, plays a neuroprotective role in a mouse model of PD. In the current study I examined the effect of NaB treatment on the 6-hydroxydopamine (6-OHDA) rat model of PD. The results show that rats who had NaB (150-300 mg/kg/day) in their water continuously beginning one week before the 6-OHDA lesion developed significantly less L-DOPA-induced dyskinesia (LID) as measured by the Abnormal

Involuntary Movements (AIMs) scale after an acute injection of L-DOPA (25 mg/kg) and benserazide (15 mg/kg, i.p.) than rats who had regular water. Also, the progression of LID between the first and second injections of L-DOPA was significantly lower in the group receiving NaB during the period between the injections than in the group that received regular water. To assess limb functionality and Parkinsonism, I measured the performance of both groups on the stepping test. The NaB-treated group performed significantly better, indicating a possible neuroprotective or therapeutic effect of NaB. Finally, while previous findings were ambiguous about this subject, I show that LID due to high doses of L-DOPA correlates very well with the number of amphetamine (5 mg/kg, i.p.)-induced rotations in untreated 6-OHDA rats, but not in NaB rats.

Disclosures: **M.K. Shammas:** None. **R.T. Kennedy:** None.

Poster

414. Neurodegeneration and Neuroprotection in Parkinson's Disease

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Program#/Poster#: 414.20/T12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BRACE grant

grant from Northcott Devon Medical Foundation

Plymouth University

Title: Distribution of beta-synuclein in dementia with Lewy bodies brain tissues

Authors: T. EVANS, K. COWAN, *O. ANICHTCHIK;
Plymouth Univ., Plymouth, United Kingdom

Abstract: Dementia with Lewy bodies (DLB) is the second most frequent neurodegenerative dementia characterised by accumulation of aggregated fibrils of α-synuclein in limbic areas and forebrain. Synucleins (α-, β-, γ-) are small pre-synaptic proteins with a similar distribution in the brain however, unlike α-synuclein, β-synuclein is not thought to have aggregation properties and has been shown to prevent α-synuclein aggregation *in vitro* and *in vivo*. Regional changes in β-synuclein expression levels, in addition to observations of structural synaptic alternations and small α-synuclein aggregates, have been observed in the brains of DLB patients suggesting pre-synaptic changes may be among events preceding neuronal degeneration. We have shown previously that α-synuclein aggregation causes early synaptic dysfunction in the mouse model of Parkinson’s disease (PD), and morphological disorganisation of synaptic proteins in post-mortem

tissues of early PD patients. Here we were asking whether α-synuclein-associated synaptic protein disorganisation is also present in DLB, and whether β-synuclein distribution in DLB brain is altered in relation to the α-synuclein aggregation burden. We have examined frontal, occipital cortex and the hippocampus in patients with DLB with less than 5 years disease duration and age-matched controls. Tissues were obtained from the Human Brain Bank of Parkinson's disease (UK) and from Brains for Dementia Research Brain Banks (UK). Immunohistochemical staining for full length and oligomeric α-synuclein, β-synuclein, SNARE proteins (synaptobrevin/VAMP2, SNAP25, syntaxin) and lysosomal markers were performed on paraffin-embedded sections, and semi-quantitative Western blotting was done to examine changes in protein levels. We have identified close association between β-synuclein and SNARE proteins in the cortical areas, but not in the hippocampus. In addition, we have detected an increase of β-synuclein levels in the frontal cortex, while this was reversed in the occipital cortex. The pilot data indicated that levels of β-synuclein were inversely correlated with levels of oligomeric α-synuclein. These findings will be discussed in relation to the pathophysiology of synaptic dysfunction in DLB.

Disclosures: **T. Evans:** None. **K. Cowan:** None. **O. Anichtchik:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.01/T13

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS081118

Michael J Fox Foundation

Title: Stimulation amplitude-dependent modulation of neuronal activity around chronically implanted thalamic deep brain stimulation arrays

Authors: ***M. D. JOHNSON**, Y. XIAO;
Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: There is a strong clinical need for implantable deep brain stimulation (DBS) lead designs that allow for better steering of electric fields within the brain, especially in cases where stimulation through a misplaced DBS lead results in low-threshold side-effects. Radially segmented DBS arrays (DBSAs) have potential to rescue therapy in such cases, but the electrophysiolgical effects of directing current within the brain through these arrays remain

relatively unexplored. In this study, we investigated how stimulation amplitude affects neuronal firing rate and pattern changes around a motor thalamic DBSA. A DBSA with 8 rows of 4 radial electrodes was implanted unilaterally in the motor thalamus in two non-human primate. A single row of electrodes was selected where each contact performed 3 sets of 100Hz stimulations at 350μA, 250μA and 150μA, while microelectrode recordings were made in a grid pattern around the DBSA. Peri-stimulus time histograms were compared between DBS and pre-DBS periods (30-60s) and grouped into nine categories based on firing pattern modulation (FPM) and change in firing rate. An entropy-based method was used to quantify the degree of FPM and determine instances of significant modulation. For both thalamic DBSA implants, thalamic neurons sampled around the lead showed firing pattern and rate modulation that were sparsely distributed and were not confined to regions in the immediate proximity of the DBSA. Higher stimulation amplitudes resulted in greater change in firing pattern (i.e. increased regularity) in the subgroup of cells that showed significant FPM under at least one stimulation amplitude. Interestingly, only 3.25% (±3.8%) of DBS pulses produced a phase-locked spike in cells with a significant excitatory FPM. While computational models often predict uniform modulation of neuronal spike activity around a DBS lead, this study demonstrates that the volume of neuronal modulation is in fact sparsely represented. Moreover, while neuronal activity becomes time-locked to DBS, only a small fraction of stimulus pulses actually result in a phase-locked spike. This work will help inform the next generation of computational models of DBS and provide insight for future implant designs.

Disclosures: **M.D. Johnson:** None. **Y. Xiao:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01 NS037019

University of Minnesota’s MnDRIVE (Minnesota’s Discovery, Research and Innovation Economy) initiative. Postdoctoral Fellowship in Neuromodulation

Title: Effect of parkinsonism and deep brain stimulation on neural oscillations and phase-amplitude coupling within the motor cortex, subthalamic nucleus and globus pallidus

Authors: *D. ESCOBAR SANABRIA[1], L. A. JOHNSON[1], J. ZHANG[1], S. NEBECK[1], M. D. JOHNSON[2], G. F. MOLNAR[1], K. B. BAKER[1], J. L. VITEK[1];
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Abstract: A neurophysiological biomarker that reflects Parkinson's disease (PD) severity or motor symptoms is still being debated. Alterations in neural oscillations across sites in the basal ganglia-thalamocortical network and in various low- and high-frequency bands have been implicated in the pathophysiology of PD and therapeutic mechanisms of deep brain stimulation (DBS). Recently, elevated coupling between the phase of low-frequency and the amplitude of high-frequency neural oscillations (phase-amplitude coupling, PAC) has been hypothesized to be a biomarker of PD severity and symptoms. It is not well understood, however, how oscillatory activity and PAC are altered from the normal to the parkinsonian condition, within different structures of the motor network, and during therapeutic DBS in different targets. In an effort to understand these alterations, we characterized oscillatory activity and PAC within the subthalamic nucleus (STN), globus pallidus (GP), and motor cortex (M1) of two rhesus macaques in the normal and parkinsonian conditions, and during therapeutic DBS. Each animal was implanted with a DBS lead in both STN and GPi and a Utah array in M1. The animals were rendered parkinsonian by systemic administration of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). We analyzed data collected in the awake resting state and assessed both static measures and the temporal dynamics of oscillatory activity and PAC. Analysis showed that oscillations in single frequency bands within the studied brain structures did not differ between normal subjects. In the parkinsonian state, however, we observed significant changes in oscillatory activity and PAC which varied across animals, suggesting there may be subject-specific signatures of network dysfunction. In particular, PAC emerged in the M1 (~25 Hz for phase, ~50 Hz for amplitude) of one subject but in the GP (~10 Hz for phase, ~200 Hz for amplitude) of the other. In the first subject, STN-DBS suppressed PAC in M1 but GPi DBS did not, whereas in the second subject, GPi-DBS suppressed PAC in GP but STN-DBS enhanced it. These results suggest that PAC may manifest in different structures of the basal ganglia-thalamocortical network in the parkinsonian condition. This study also highlights that the relationship between PAC and parkinsonian motor signs and the role of PAC in mediating the therapeutic effects of DBS remain unclear.

Disclosures: D. Escobar Sanabria: None. **L.A. Johnson:** None. **J. Zhang:** None. **S. Nebeck:** None. **M.D. Johnson:** None. **G.F. Molnar:** None. **K.B. Baker:** None. **J.L. Vitek:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Program#/Poster#: 415.03/T14

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01 5r01ns077657-04

Title: Neuro-pathophysiology of SMA-M1 single unit activity during visually guided reach in the progressive MPTP nonhuman primate model of Parkinson's disease

Authors: *B. CAMPBELL[1], C. M. HENDRIX[1], B. J. TITTLE[1], Y. ADIBI[1], Z. M. WEINSTOCK[2], G. F. MOLNAR[1], M. D. JOHNSON[3], K. B. BAKER[1], J. L. VITEK[1]; [1]Neurol., [2]Col. of Biol. Sci., [3]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Motor signs in Parkinson's disease (PD) are primarily related to pathophysiological changes in basal ganglia thalamo-cortical (BGTC) motor circuitry affecting the kinematics of visually guided movement. However, the underlying mechanisms and changes in cortical neural activity with disease severity are not well understood. This study characterized task specific changes in behavior and neuronal activity in SMA and M1 during a visually guided movement in the MPTP NHP. Concurrent MER, (paired-SU within and across M1-SMA cortical sites) and task behaviors, (reach kinematics and eye movements) were recorded. Compared to the normal state, eye movements in the parkinsonian state showed an increase in the occurrence and duration of eye fixation prior to presentation of the visual go-cue and followed by an increase in time-locked saccades towards the visual target after the go-cue. In contrast, initiation of hand movement after go-cue was delayed in the parkinsonian state. In addition, the reach kinematics were also impaired resulting in longer and more variable reach times, decreased accuracy, and degraded stereotypy in reach paths. Perievent histograms, auto-correlograms, and cross-correlograms of SU activity time-locked to task behaviors showed a decrease in event related modulation and correlated paired-SU activity (both magnitude and timing). Kinematics of visually guided reach behavior and related cortical activity presented task specific changes in the parkinsonian state. Saccade initiation towards the target were faster and closely time-locked to the onset of the go-cue and may reflect difficulty in suppressing reflexive saccades towards external visual cues. Reach onset, on the other hand, was delayed relative to the go-cue. Disinhibition of saccadic movement coupled with prolonged reaction times may reflect a compensatory strategy of engaging sequential, stepwise mechanisms during the visuomotor reach. Finally, task related cortical activity in M1 and SMA also changed in PD resulting in a reduction in task related modulation and increased variability in timing relative to task events. A decrease in time-locked neural modulation in PD coupled with degradation in overall visuomotor coordination is consistent with a model of PD dysfunction that accounts for changes in selective firing patterns, suppression of non-selective firing patterns, and the temporal relationships of neural activity within cortical-cortical and across the thalamo-cortical motor pathways.

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Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS081118

Michael J Fox Foundation

Title: Improved spatial resolution of local field potentials with deep brain stimulation arrays in the globus pallidus and subthalamic nucleus

Authors: *S. ZHANG, M. D. JOHNSON;
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Abstract: The basal ganglia nuclei are known to have a functional topography composed of motor subcircuits and oscillatory networks that are likely to be critically important for the successful application of closed-loop deep brain stimulation (DBS) therapy to treat Parkinson's disease. To accurately measure oscillatory dynamics using local field potentials (LFPs), the bipolar pair of recording electrodes should match the geometry of the target neural dipoles so that the electrodes do not shunt the underlying heterogeneity of sinks and sources. We hypothesized that there is spatially heterogeneous oscillatory activity in the globus pallidus (GPe/GPi) and subthalamic nucleus (STN) at a spatial scale smaller than that observable with conventional clinical DBS leads. In this study, DBS arrays (DBSAs), with 8 rows of 4 radially-oriented electrodes, were chronically implanted in the GPe/GPi and in the STN in a non-human primate. Electrodes on the GPe/GPi (and STN) arrays were 360μm x 470μm (350μm x 360μm) with axial pitch given by 572 μm (750 μm) and circumferential pitch given by 471 μm for both arrays. Resting-state LFP recordings were collected across both arrays, filtered in the low beta band (12-20 Hz), and current source density was calculated. Multiple oscillatory dipoles were found across both DBS arrays. Notably, when simulating LFP recordings through a conventional DBS lead with four virtual cylindrical macroelectrodes - i.e. by shorting two rows of electrodes along the DBS array and taking the differential between shorted row pairs - oscillatory dipoles were not observed in the STN and two oscillatory dipoles were found for the GPe/GPi lead near the border between nuclei. Additionally, the differential LFP signal amplitude was 4x larger between individual electrodes around or along the DBS array than in the case of the differential signal between two virtual macroelectrodes. Together, the data suggest that closed-loop DBS will benefit from using DBS leads that consist of a more spatially refined distribution of electrodes along and around the lead shank.

Disclosures: S. Zhang: None. **M.D. Johnson:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant R01 NS077657-02

Title: Low frequency modulations in the Supplementary Motor Area (SMA) and motor cortex (MC) in the MPTP nonhuman primate (NHP) model of Parkinson's disease.

Authors: *Y. ADIBI[1], C. HENDRIX[2], B. CAMPBELL[2], K. BAKER[2], J. L. VITEK[2];
[2]Neurol., [1]Univ. of Minnesota, Minneapolis, MN

Abstract: Changes in the basal ganglia thalamocortical motor circuit are thought to underlie the motor signs in Parkinson's disease. Although multiple studies have reported changes that occur in subcortical structures, little is known about changes in cortical neuronal activity. To further explore the changes that take place in these cortical motor areas, we recorded local field potentials (LFPs) in SMA and M1 in both normal and parkinsonian conditions. Oscillatory activity was analyzed through spectrograms during epochs of a controlled 8-target center-out reaching task. Spectrograms were aligned with the visual go-cue and averaged across trials. Low frequency (0-8 Hz) power increased in SMA and M1 following the go-cue in both conditions. Changes in power however, showed different patterns in the normal and parkinsonian state. Beta (13-30 Hz) desynchronization immediately followed the visual go-cue and was relatively more persistent in the naïve state as compared to PD. Furthermore, an increase in beta activity was observed following target touch coincident with upper limb freezing in the return movement. Differences in the patterning, manner of activation, and timing of return of LFP activity in the different frequency spectrums between the two cortical regions may be suggestive of area-specific biomarkers and indicative of cortical activity changes underlying the development of Parkinson's disease.

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Poster

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NIH Grant NS077657

MnDRIVE Neuromodulation Postdoctoral Fellowship

Parkinson's Disease Foundation Postdoctoral Research Fellowship

Title: Evolution in the modulation of passive responses in primary motor cortex during prolonged STN DBS in the parkinsonian monkey

Authors: *J. WANG[1], S. NEBECK[1], L. A. JOHNSON[1], J. ZHANG[1], M. D. JOHNSON[2], K. B. BAKER[1], G. F. MOLNAR[1], J. L. VITEK[1];
[1]Neurol., [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Deep brain stimulation (DBS) in the subthalamic nucleus (STN) has been a therapeutic treatment for Parkinsonian motor symptoms for decades. However, the effect of STN DBS on cell activity in the primary motor cortex (M1), a key nodal point in the basal ganglia-thalamo-cortical network, is not well understood. Moreover, the alteration of this neuronal activity during long-term STN DBS has not yet been explored. In the present study, the M1 neuronal responses to passive manipulation of arm during prolonged STN DBS were studied in the MPTP non-human primate (NHP) model of Parkinson's disease.
A NHP (female, 6kg) was implanted with an 8-contact scaled-down DBS lead in the STN and a 96-channel Utah array in the arm area of M1. Optimal stimulation contacts and parameters (0.2mA, 130Hz, 125μs) were determined by standard practice. Prolonged (4 hours) STN DBS was applied and the modified UPDRS (mUPDRS) score was assessed to confirm the efficacy of the DBS. Passive manipulations of elbow extension-flexion were performed pre, at multiple times during and post DBS. Single unit activities recorded from the array were sorted and analyzed to investigate the modulation of passive responses.
A 30-35% improvement of mUPDRS was observed during the prolonged DBS. A total of 53 well isolated single units were sorted from the array data. The modulation of passive responses in 91% of the units was altered by STN DBS. In the pre DBS baseline, 87% of the units were responsive to passive manipulation. During DBS, we observed changes in both the proportion of units responsive to passive manipulation and the depth of the modulation, and differences were observed between acute (minutes after DBS onset) and chronic changes. The number of

responsive units was slightly reduced immediately following onset of DBS. However, the proportion of units responsive to the passive manipulation increased to 94% at the end of the prolonged DBS. Differences between acute and chronic effects by DBS was also observed in the changes of modulation depth in ~30% of the units whose modulation of passive responses were altered by DBS. Overall, the modulation depths didn't increase until around one hour after DBS onset. The modulation of most of the units returned to the pre DBS level minutes after the DBS cessation.
The evolution of the modulation in the M1 neuronal responses to passive manipulation and the differences between the acute and chronic changes in modulation during the prolonged STN DBS observed in this study provide further understanding of the mechanism of DBS, as well as potential guidance to the selection of stimulation duration in future studies.

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Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 415.07/T18

Topic: C.03. Parkinson's Disease

Support: NIH grant R01 NS037019

Title: Effect of levodopa on neuronal activity in primary motor cortex in the MPTP non-human primate model of Parkinson's disease

Authors: S. D. NEBECK[1], *L. A. JOHNSON[1], J. ZHANG[1], D. ESCOBAR[1], M. D. JOHNSON[2], G. MOLNAR[1], K. B. BAKER[1], J. L. VITEK[1];
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Abstract: Primary motor cortex (M1) is a key component of the basal ganglia-thalamo-cortical (BGTC) network and likely plays a significant role in the manifestation of motor symptoms in Parkinson's disease (PD), yet relatively few studies have characterized the firing patterns of M1 neurons in PD. M1 is not only responsible for controlling voluntary limb movement, but is also integral to sensory processing of limb position and movement. It is believed that the loss of dopaminergic cells in PD disrupts this kinesthetic information processing leading to broadened receptive fields of cells throughout the BGTC network. In this study we examined the effect of levodopa, the most common medication used for treatment of PD symptoms, on neuronal

activity in M1 of two non-human primates (NHPs) rendered parkinsonian by systemic injections of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A 96-channel microelectrode array was chronically implanted in the arm area of M1 to record populations of single units, and receptive fields were characterized by changes in firing rate in response to passive manipulation of the shoulder, elbow, and wrist joints. Recordings were made before and after levodopa administration. We hypothesized that dopaminergic therapy would serve to narrow receptive fields; that is, levodopa would reduce the number of joints to which a given neuron responds and improve sensory processing. Interestingly, spontaneous firing rates did not significantly change after levodopa. Analysis was further restricted to cells that were significantly modulated by manipulation of at least one joint in the on or off levodopa condition (NHP K: 71/75, NHP J: 53/71). Levodopa narrowed receptive fields (reduction in the number of joints to which cells responded) in 52% and 42% of the cells in NHPs K and J, respectively. Moreover, the degree of firing rate modulation and temporal dynamics of the response to a given joint manipulation differed between the on and off drug state, though the details of these differences were heterogeneous across the population. In summary, levodopa therapy that improves parkinsonian motor signs leads to narrowed receptive fields together with complex changes in M1 neuronal responses to passive joint articulation, supporting the hypothesis that kinesthetic information processing in M1 is disrupted in PD and improved by dopaminergic medication.

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Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.08/U1

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

Title: STriatal-Enriched protein tyrosine Phosphatase (STEP) inhibitor TC-2153 improves aspects of cognitive dysfunction in aged Parkinsonian monkeys

Authors: *J. S. SCHNEIDER[1], C. WILLIAMS[1], P. LOMBROSO[2];
[1]Dept Pathol, Anat. & Cell Biol, Thomas Jefferson Univ., Philadelphia, PA; [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract: Although Parkinson's disease (PD) is primarily a motor disorder, cognitive impairment is a common feature that can be present at all stages of the disease. Cognitive deficits in PD patients may be associated with dysfunction of multiple cortical and subcortical neurotransmitter systems and functional circuits, that include DAergic and non-DAergic systems. Thus, the benefits of DAergic replacement therapy on PD-related cognitive deficits have been inconsistent and alternative pharmacological targets and therapies are needed to better address cognitive dysfunction in PD patients. One such potential therapy involves modulation of STriatal-Enriched protein tyrosine Phosphatase (STEP). Elevated levels of STEP are associated with cognitive deficits in a variety of disorders and lowering STEP expression either genetically or pharmacologically significantly reduced cognitive deficits in aged rodents with cognitive impairment and in transgenic Alzheimer's disease models. We recently also observed significantly increased expression of STEP in hippocampus of aged rhesus macaques with cognitive impairment as well as a significant increase in STEP expression in the pre-frontal cortex of chronic MPTP-treated monkeys that expressed fronto-striatal cognitive deficits. In this study, we examined the extent to which the STEP inhibitor TC-2153 (3.75, 7.5, and 15 mg/kg) would attenuate cognitive impairments in aged rhesus monkeys previously exposed to the neurotoxin MPTP. Six males (approx. 26 years old) with prior chronic low dose MPTP exposure and with mild motor deficits and various cognitive deficits were used. TC-2153 had no effect on performance of a continuous performance attention task or a spatial working memory task (self ordered spatial search). In contrast, TC-2153 significantly decreased the number of trials needed to learn discrimination reversals in a compound discrimination learning task and improved new learning and memory of a discrimination learning-long term retrieval task. Administration of TC-2153 resulted in animals learning new discriminations significantly faster ($P < 0.01$) than during vehicle only sessions and long-term retention was significantly better ($P < 0.05$) if the learning occurred during TC-2153 administration. These results suggest that in this model, TC-2153 may not act as a general cognition enhancer but may improve new learning and recall of that newly learned information. Supported by the Michael J. Fox Foundation.

Disclosures: **J.S. Schneider:** None. **C. Williams:** None. **P. Lombroso:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Intracerebral delivery of induced pluripotent stem cell-derived neurons using real-time intraoperative mri

Authors: *S. C. VERMILYEA[1,2], J. LU[3], M. E. OLSEN[4], Y. TAO[3], S. GUTHRIE[2], E. M. FEKETE[5], M. K. RIEDEL[5], K. BRUNNER[2], C. BOETTCHER[2], V. BONDARENKO[2], E. BRODSKY[4], W. F. BLOCK[4,6], A. ALEXANDER[2,3,4,5], S.-C. ZHANG[3,7], M. E. EMBORG[1,2,4]; [1]Neurosci. Training Program, [2]Wisconsin Natl. Primate Res. Ctr., [3]Waisman Ctr., [4]Med. Physics, [5]Psychiatry, [6]Biomed. Engin., [7]Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Induced pluripotent stem cell (iPSC)-derived neurons present an opportunity for cell replacement strategies for neurodegenerative disorders such as Parkinson's disease (PD). Improvement in cell graft targeting, distribution and density can be key for disease modification. Our team has previously developed a MRI-compatible brain port system that we have demonstrated to be successful for intraoperative MRI (IMRI) delivery of viral vectors for gene therapy strategies. The aim of this study was to develop procedures suitable for real-time IMRI (RT-IMRI) targeting guidance to facilitate its application for intracerebral delivery of biologics, especially cells, as it decreases the targeting time. Dopamine neuroprogenitor neurospheres were used for these studies. We first performed a set of *in vitro* experiments to tailor the delivery hardware (e.g. cannula) and define a range of parameters to be applied (e.g. maximal timespan allowable between cell loading in the system and intracerebral injection) to ensure cell survival. Evidence of cell death, damage to neurospheres, and obstruction of the cannula were not observed after the cells were loaded and remained in the system for up to 2 hours; some cell-settling occurred, observed as the expelling of cylindrical neurosphere aggregates that could be easily dissociated. To assess if the process affects cell identity, a subset of the cells were plated after passing through the system and allowed to differentiate for 2 weeks. Immunostaining against tyrosine hydroxylase and βIII-tubulin confirmed that the dopaminergic identity of the cells was unchanged. Next we performed cell injections into a 0.6% agarose gel as a brain surrogate, to analyze patterns of cell distribution and associated intra-line pressure changes. We found that multiple distinct deposits could be easily delivered along the cannula tract and that the pressure reflected each bout of cell expulsion. Lastly, we evaluated the feasibility of applying the RT-IMRI system to deliver cells into the nonhuman primate brain, by injecting neurospheres labeled with Hoechst into the putamen nucleus. Experimental procedures were approved by the IACUC of the University at the Wisconsin-Madison. All efforts were made to minimize the number of animals used and to ameliorate any distress. Postmortem evaluation 2 weeks later showed abundant cell survival of Hoescht-positive cells that expressed nestin. Our results demonstrate that the RT-IMRI delivery system provides valuable guidance, monitoring, and visualization during intracerebral iPSC-derived neuron delivery that is also compatible with cell survival.

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Poster

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Office of the Vice Chancellor for Research and Graduate Education, University of Wisconsin - Madison

Wisconsin National Primate Research Center, University of Wisconsin - Madison

Department of Medical Physics, University of Wisconsin - Madison

Title: Positron emission tomography imaging of cardiac neuroprotection induced by peroxisome proliferator-activated receptor gamma (PPARγ) activation

Authors: *J. SHULTZ[1,2], H. RESNIKOFF[1], V. BONDARENKO[1], J. HOLDEN[3], T. BARNHART[3], P. LAO[3], B. CHRISTIAN[3], J. NICKLES[3], C. F. MOORE[4], M. EMBORG[1,2,3]; [1]Wisconsin Natl. Primate Res. Ctr., [2]Cell. and Mol. Pathology Grad. Program, [3]Dept. of Med. Physics, [4]Dept. of Psychology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Loss of postganglionic sympathetic innervation to the heart is a characteristic pathology of cardiac dysautonomia in Parkinson's disease (PD); disease-modifying strategies are not available. Neuronal loss in PD is associated with inflammation and oxidative stress and can be mimicked in animals by dosing of the catecholaminergic neurotoxin 6-hyroxydopamine (6-OHDA). Interestingly, activation of the peroxisome proliferator-activated receptor gamma (PPARγ) decreases inflammatory response (microglial, macrophage) and production of reactive oxygen species. Here we report our studies with the PPARγ agonist pioglitazone in a nonhuman

primate model of cardiac dysautonomia induced by systemic dosing of 6-OHDA. We aimed to assess the neuroprotective efficacy of pioglitazone using positron emission tomography (PET) with radioligands specific to catecholaminergic innervation ([11C]meta-hydroxyephedrine, MHED), inflammation ([11C]peripheral benzodiazepine receptor 28, PBR28), and oxidative stress ([61Cu]diacetyl-bis(N(4))-methylthiosemicarbazone, ATSM). Ten adult, male rhesus monkeys received intravenous 6-OHDA (50mg/kg) and 24 hours later were randomly assigned to begin daily oral dosing of placebo (n=5) or pioglitazone (5mg/kg; n=5). At baseline, 1 and 12 weeks post-6-OHDA all animals were PET scanned with MHED, PBR28 and ATSM; 24 hours after the final PET scan animals were euthanized by trans-aortic perfusion and heart tissue collected. One week post-neurotoxin, MHED uptake was dramatically reduced in all animals compared to baseline (placebo 86% decrease; pioglitazone 82%). Concurrently, PBR28 uptake was attenuated in the pioglitazone-treated animals compared to placebo (ANOVA $p=0.007$), with the difference most pronounced in the base of the heart. Preliminary analysis of ATSM data also suggests decreased uptake at one week in pioglitazone-treated animals. By twelve weeks post-neurotoxin, partial recovery of MHED uptake was significantly greater in the pioglitazone (54% Retention Deficit (%RD)) animals compared to placebo (70 %RD; ANOVA $p<0.05$), with greatest preservation in the apical, septal left ventricle, while PBR28 and ATSM uptake values returned to baseline levels. Ongoing immunohistochemical quantification of panneuronal marker PGP9.5 and catecholaminergic marker tyrosine hydroxylase (TH) in left ventricle tissue supports PET findings, suggesting preservation of sympathetic nerves in the heart induced by pioglitazone. Overall, these results demonstrate PPARγ-activation induced cardiac sympathetic neuroprotection in association with decreased inflammation and oxidative stress.

Disclosures: **J. Shultz:** None. **H. Resnikoff:** None. **V. Bondarenko:** None. **J. Holden:** None. **T. Barnhart:** None. **P. Lao:** None. **B. Christian:** None. **J. Nickles:** None. **C.F. Moore:** None. **M. Emborg:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.11/U4

Topic: C.03. Parkinson’s Disease

Support: NIH Grant P51OD011106

NIH Grant R24OD019803

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NIH Grant DC011130

Department of Surgery at University of Wisconsin-Madison

Title: Early development of common marmoset vocalizations

Authors: *C. A. JONES[1,2,3,5], M. K. DUFFY[5], S. A. HOFFMAN[5], N. J. SCHULTZ-DARKEN[5], K. M. BRAUN[5], M. R. CIUCCI[2,1,3], M. E. EMBORG[3,5,4];
[1]Surgery - Div. of Otolaryngology, [2]Communication Sci. and Disorders, [3]Neurosci. Training Program, [4]Med. Physics, Univ. of Wisconsin - Madison, Madison, WI; [5]Wisconsin Natl. Primate Ctr., Madison, WI

Abstract: Patients with Parkinson's disease (PD) are known to have considerable voice and speech impairments that begin to manifest early in the disease progression. The common marmoset (*Callithrix jacchus*) is a target species for PD model development. These New World monkeys are ideal for evaluating vocalizations, as they are social animals who generate a variety of call types without complex elicitation protocols. The purpose of this project is to establish how common marmoset vocalizations change with development in order to understand how and when communication might change in a common marmoset model of PD. Fourteen developing marmosets were longitudinally video- and audio-recorded between the ages of 1-149 days in a naturalistic setting without any vocalization elicitation protocol. Vocalizations were coded for call type (cry, tsik, trill, phee, and trill-phee) and analyzed for duration (sec), minimum and maximum frequency (Hz), and bandwidth (Hz). Mixed model linear regressions were performed to assess the effects of age on call parameters listed above. Tsik bandwidth decreased with age ($p=0.004$) while tsik minimum and maximum frequency increased ($p=0.01$; $p=0.012$). Phee and trill-phee bandwidth also decreased with age ($p=0.04$; $p=0.01$). Call duration did not change with age ($p\geq0.05$). Common marmoset vocalization development is call type-dependent, with tsik, phee, and trill-phee calls changing most with age. Decreases in call bandwidth may represent an improvement in motor control for producing complex calls. This dataset can serve as a comparison group for future studies involving transgenic models of neurological diseases, with the capability for investigation into early changes to vocal function associated with PD pathology and into voice and speech treatment modalities.

Disclosures: C.A. Jones: None. **M.K. Duffy:** None. **S.A. Hoffman:** None. **N.J. Schultz-Darken:** None. **K.M. Braun:** None. **M.R. Ciucci:** None. **M.E. Emborg:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NSF CAREER CBET-1351692

NSF IGERT DGE-1250104

HFSP Young Investigators Award, RGY0088

Title: Wirelessly programmable module for custom deep brain stimulation

Authors: *E. M. LEWIS[1], C. KEMERE[2];
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Abstract: Typical experiments in rodent models of Parkinson's disease rely on a tether to deliver deep brain stimulation (DBS) to the implanted electrode. Tethered studies make long-term stimulation and complex behavior study difficult or impossible. While tether-free rodent-scale pulse generators were developed to address this need, they lack the flexibility required to easily test different patterns of stimulation. We developed a wirelessly programmable stimulator and a customizable 3-D printed housing that allow for chronic delivery of normal or novel patterns of DBS in rats. The housing weighs 16.5 g and is: 35 x 26 x 34 mm (l, w, h). The stimulator utilizes an embedded microcontroller to generate biphasic current pulses to a concentric electrode in the subthalamic nucleus. The amplitude of stimulation ranges from 30 to 110 μA, at a frequency of 50 to 200 Hz with a pulse width from 30 to 90 μs. As an additional feature, we are adding the ability to optionally introduce a random offset to the inter-pulse interval. We have previously shown that such jitter, which can vary from 1 to 4 μs, may have therapeutic benefit. These programming settings can be changed wirelessly through near field communication (NFC) with an Android mobile application. We tested our device in vivo in hemiparkinsonian rats (unilaterally-lesioned using injections of 6-OHDA), assessing rotational behavior following amphetamine administration. We found similar behavioral responses to stimulation using a traditional tethered stimulator and our untethered unit. The results of this approach provide a cost effective method for future high throughput studies in rodent DBS. Thus, we anticipate that our open source platform will provide tremendous value to our experiments and to future studies involving chronic stimulation in rodent disease models.

Disclosures: E.M. Lewis: None. **C. Kemere:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.13/U6

Topic: E.03. Basal Ganglia

Support: DFG Grant KFO 247

Prof. Klaus Thiemann Foundation, Thiemann Fellowship

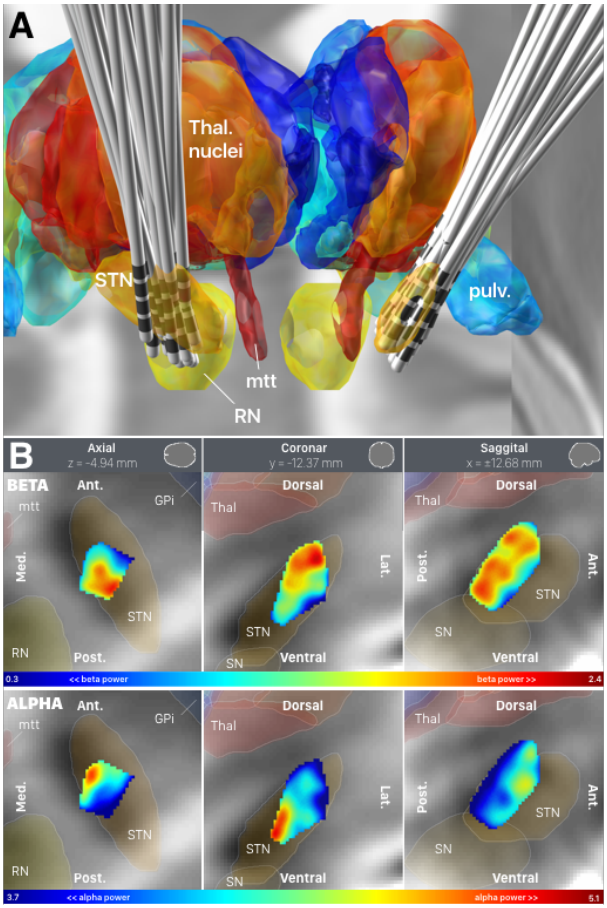
Stiftung Charité, Max Rubner Prize

Berlin Institute of Health, Clinical Scientist Program

Title: Toward an electrophysiologically defined “sweet spot” for deep brain stimulation within the subthalamic nucleus

Authors: *A. HORN[1], W.-J. NEUMANN[1], K. DEGEN[1], G.-H. SCHNEIDER[2], A. KÜHN[1]; [1]Neurol. Dept., [2]Neurosurg. Dept., Charité – Univ. Med., Berlin, Germany

Abstract: Background: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) in patients with Parkinson‘s disease (PD) is a well established therapy with excellent clinical results. A potential physiomarker of motor symptoms in PD is enhanced power of local field potential (LFP) activity in the beta band[1] and its spatial source could potentially be used to refine DBS targeting. The aim of this study was to map the distribution of beta power from a large cohort of patients onto anatomical space.
Methods: In total, 62 patients
(42 male; mean age 61 ± 9 yrs) that underwent DBS surgery for severe Parkinson's disease (PD) between 2000-2014 were included. Patients received two quadripolar electrodes (Medtronic 3389). Pre- and postoperative MRI were normalized into standard space using DARTEL. Electrode placement was localized using Lead-DBS[2]. LFP recordings were acquired at rest after overnight withdrawal of dopaminergic medication within a postoperative interval of 2-7 days from externalized leads. Power in alpha (8-12 Hz) and beta (13-35 Hz) frequency bands were calculated between adjacent contacts.
Results: Analysis of electrode localizations located 364 of 432 electrode contacts (84%) inside the STN defined by the Morel atlas[3] (Fig. 1A). The peak of the final beta-mapping resided within the postero-dorso-lateral portion of the STN at x = ±12.0 y = -14.4 z = -8.6 mm whereas the alpha-peak was located antero-ventro-medial to it at x = ±11.5, y = -12.5, z = -7.9 mm (Fig 1 B). Our findings suggest a spatial distribution of alpha and beta sources within the STN, the latter highly corresponding to its sensorimotor functional zone.
References: 1. Brown, P. Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson's disease. *Mov. Disord.* (2003). 2. Horn, A. & Kühn, A. A. Lead-DBS: a toolbox for deep brain stimulation electrode localizations and visualizations. *NeuroImage* (2015). 3. Krauth, A. *et al.* A mean three-dimensional atlas of the human thalamus: generation from multiple histological data. (2010).



Disclosures: **A. Horn:** None. **W. Neumann:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. **K. Degen:** None. **G. Schneider:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic, St. Jude Medical, Boston Scientific. **A. Kühn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); St. Jude Medical, Medtronic, Ipsen Pharma, Boston Scientific.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 415.14/U7

Topic: E.03. Basal Ganglia

Support: DFG, KFO 247

Stiftung Charité, Max-Rubner-Preis

Berlin Institute of Health, Clinical Scientist Program

Professor Klaus Thiemann Foundation, Thiemann Fellowship

Title: Three-dimensional definition of two prominent deep brain stimulation targets based on a multimodal high-definition MNI template

Authors: *S. EWERT[1], A. HORN[1,2];
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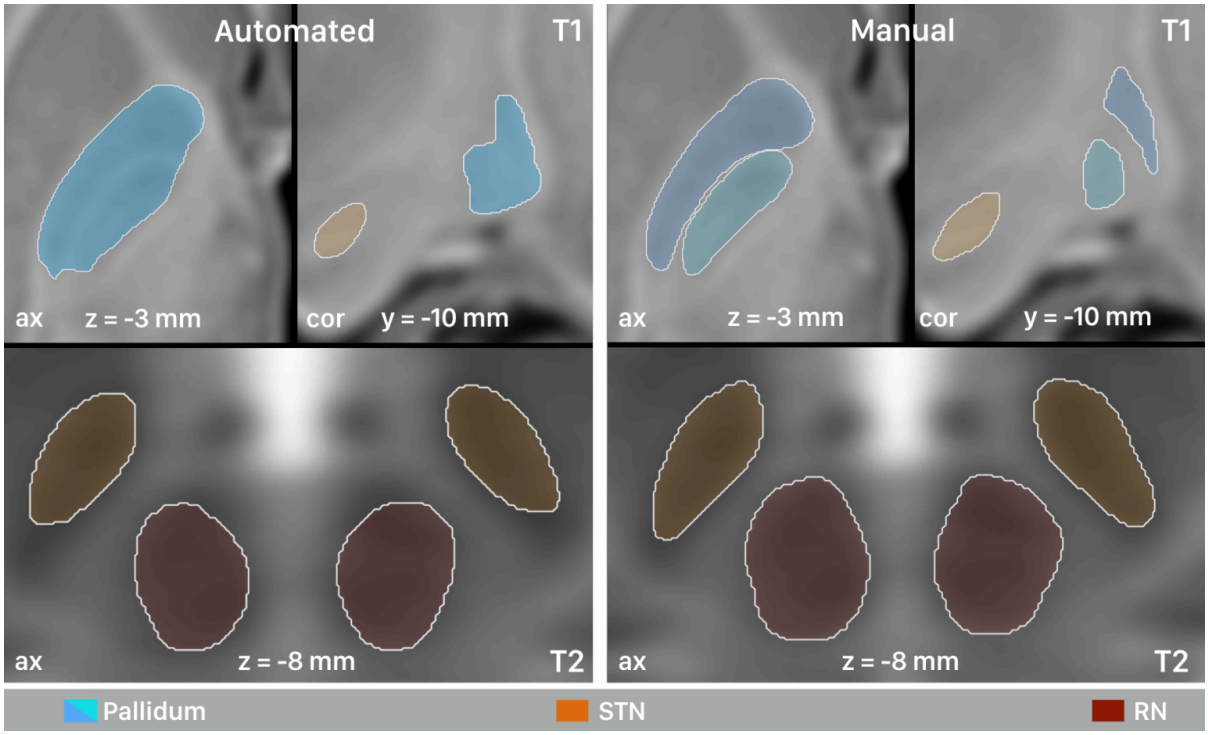
Abstract: Background: Deep brain stimulation (DBS) of target structures in patients with Parkinson‘s disease and Dystonia is a well-established therapy. To further optimize this therapy, an evaluation of electrode placement relative to their target structure is essential. To do so, accurate volumetric atlases defining the exact locations of DBS targets in a standardized stereotactic space are needed. Here, we present an atlas that precisely defines the primary two DBS targets, the subthalamic nucleus (STN) and the internal part of the pallidum (GPi) within MNI space.
Methods: An observer independent three-level algorithm was designed to robustly segment subcortical DBS target structures (GPe, GPi, Red nucleus (RN) and STN) within MNI space by simultaneously using T1, T2, proton density and T2 relaxometry versions of the ICBM 152 2009 nonlinear asymmetric template. This algorithm uses intensity values of all four modalities of each brain voxel to calculate Mahalanobis distances from priorly estimated intensity distributions of each target region. Thus, each voxel is assigned a probability to belong to each target structure based on its intensity distribution across acquisitions. Automated results were used as primary guidance to manually segment structures on a high definition version of the template following a manual segmentation protocol for subcortical structures modified from 1.
Results: The algorithm was able to robustly segment all target structures but could not differentiate GPe from GPi. The pallidum was further segmented into GPi and GPe manually. Likewise, STN and RN were re-segmented, resulting in an atlas that gives the exact location of DBS target structures within MNI space (Fig. 1). The atlas will be made available within Lead-DBS software and makes it possible to assess relationships between DBS electrodes and target structures within standard space.
References: 1. Pelzer, E.A. et al., 2013. Cerebellar networks with basal ganglia: feasibility for tracking cerebello-pallidal and subthalamo-cerebellar projections in the human brain. European Journal of Neuroscience



Disclosures: **S. Ewert:** None. **A. Horn:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.15/U8

Topic: E.03. Basal Ganglia

Support: Israel Science Foundation (ISF) 743/13

Tourette Syndrome Association (TSA)

Title: Basal ganglia disinhibition in Tourette syndrome

Authors: ***I. BAR-GAD**, M. ISRAELASHVILI;
Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Tourette syndrome (TS) has been associated with abnormalities in the cortico-basal ganglia (CBG) system, and specifically with abnormal inhibition within the striatum. Functional models of the basal ganglia (BG) emphasize the role of disinhibition of its output nucleus, the

globus pallidus internus - GPi, with the formation of different hyperkinetic disorders including TS. According to these models, global reduction of GPi activity leads to disinhibition of its thalamocortical targets resulting in cortical over-activation and the expression of hyperkinetic symptoms. Focal reduction of GPi activity, on the other hand, leads to disinhibition of local cortical networks and the release of individual movements i.e., tics. In this study we test the disinhibition hypothesis of TS by examining its neurophysiogical correlates in TS patient undergoing implantation of deep brain stimulation (DBS) electrodes and in the rodent and non-human primate (NHP) striatal disinhibition models of the disorder. We recorded the neuronal activity in: (1) Awake TS patients undergoing DBS electrode implantation in the anterior (limbic) GPi. (2) Freely behaving rodents and head restrained NHPs expressing motor tics following local micro-injection of bicuculline, a $GABA_A$ antagonist, into the sensorimotor striatum. The neuronal activity throughout the CBG pathway (globus pallidus in human patients, motor cortex, striatum & globus pallidus in the model animals) was recorded and correlated with the expression of tics. Neuronal activity in the animal models was characterized by tic related neuronal activity which encoded both the location of the tic (via striatal activity) and the timing of the tic (via cortico-striatal input). Activity in both segments of the globus pallidus of TS patients and the animal models was reduced compared to normal controls, presumably due to simultaneous increased inhibition through the direct and indirect pathways. This reduced activity leads to disinhibition of downstream targets of the BG such as the thalamus and cortex. Thus, converging evidence from TS patients and animal models of the disorder demonstrate major changes in the activity of the CBG pathway. These changes which including global and local disinhibition of BG targets are associated with both the overall state of the disorder and the expression of individual tics.

Disclosures: I. Bar-Gad: None. **M. Israelashvili:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.16/U9

Topic: C.03. Parkinson’s Disease

Support: NIH R01 MH106173

Title: Patient-specific models of local field potentials recorded from deep brain stimulation electrodes

Authors: N. MALING[1], S. F. LEMPKA[2], Z. BLUMENFELD[3], H. BRONTE-STEWART[3], *C. C. MCINTYRE[1];

[1]Dept. of Biomed. Engin., Case Western Reserve Univ., Cleveland, OH; [2]Cleveland Clin., Cleveland, OH; [3]Stanford Univ., Palo Alto, CA

Abstract: Emerging technological innovations in clinical deep brain stimulation (DBS) are enabling chronic recording of local field potentials (LFPs) from the implanted electrodes, with hopes that the signals could be useful as representative biomarkers of the patient-specific disease state. However, scientific details on the biophysical origin of these LFP signals remains elusive, and little is known about how the patient's unique brain anatomy and electrode placement impact the recording of such signals. Therefore, we developed a computational framework to theoretically analyze LFP recordings from clinical DBS electrodes that can be customized to individual patients. To demonstrate our model system, we selected a subject with Parkinson's disease implanted with a Medtronic Activa PC+S DBS system. A patient-specific reconstruction of their subthalamic nucleus (STN) and DBS electrode implant location was generated using their clinical imaging data (MRI and CT). This patient-specific anatomical model was then used to define the parameters of a finite element volume conductor model, and to dictate the locations of 200,000 STN multi-compartment cable model current sources relative to the implanted DBS electrode in an anatomically realistic way. We then used this model system to examine the impact of distributing subpopulations of highly synchronous neurons within the STN volume on the recorded LFP signal and compared those theoretical results to the experimentally measured LFPs. We found that incorporating patient-specific STN anatomy resulted in measureable changes to LFP amplitude compared to a spherical STN shape. We also found that the recording configuration and filtering effects have a substantial impact on the amplitude and frequency content of the recorded signal. Neuronal density in the area surrounding the electrode had a graded effect on LFP amplitude, while a more profound effect was generated by varying the synchrony of spatially discrete subpopulations of neurons near the electrode. Finally, we found that explicitly incorporating patient-specific details into appropriately parameterized DBS LFP models enabled us to accurately reproduce key features of the experimentally recorded signals, such as state-dependent modulation and recording location dependence of beta band activity.

Disclosures: N. Maling: None. **S.F. Lempka:** None. **Z. Blumenfeld:** None. **H. Bronte-Stewart:** None. **C.C. McIntyre:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.17/U10

Topic: E.03. Basal Ganglia

Title: Subthalamic nucleus oscillations as predictor of motor cortical excitability measured by transcranial magnetic stimulation

Authors: *K. UDUPA[1,2], A. GHAHREMANI[3], J. R. WESSEL[4], U. SAHA[2], T. HOQUE[2], A. R. ARON[5], R. CHEN[3];
[1]Krembil Res. Institute, UHN, Toronto, ON, Canada; [3]Neurology/Medicine, [2]Krembil Res. Inst., Toronto, ON, Canada; [4]Dept. of Psychological and Brain Sci., Univ. of Iowa, Iowa city, IA; [5]Dept. of Psychology, Univ. of California San Diego, La Jolla, CA

Abstract: It is well known that there is considerable trial-to-trial variation in motor evoked potential (MEP) amplitudes evoked by transcranial magnetic stimulation (TMS) of the motor cortex (M1), but the basis of this variability is not well understood. Electroencephalographic (EEG) studies have shown that higher MEP amplitudes are associated with decreased beta power in the rolandic regions and increased fronto-parietal beta band coherence about one second prior to the stimulation. Since cortical oscillations are strongly influenced by subcortical (basal ganglia-thalamocortical) networks, fluctuations in subthalamic nucleus (STN) local field potentials (LFP) could also relate to this variability. We hypothesized that higher MEP amplitudes (more motor excitability) would be associated with lower beta band power in the STN (less of an 'akinetic' signature). We studied 11 Parkinson's disease patients implanted with STN deep brain stimulation electrodes with externalized leads. Using TMS, we stimulated the left M1 (70mm, figure of eight coil, 20 trials with 5 sec between trials) to elicit 1mV MEP amplitude in the resting right first dorsal interosseous muscle. We recorded LFP oscillations in bilateral STN from deep brain stimulation electrodes and computed the LFP power before TMS (-200 to -1000 ms) in the theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30Hz) frequency ranges using bipolar montages (0-1, 1-2, 2-3). MEP amplitudes from individual trials had significant positive correlation with beta power in the left STN in seven out of 11 patients, whereas no correlation was found for the right STN. Fluctuations in theta and alpha power did not correlate with MEP amplitudes. The average MEP (20 trials) amplitude also positively correlated with amplitude of beta oscillations in left STN across patients ($r=0.58$, $p=0.04$). Our study demonstrates that spontaneous variations in STN beta oscillations may predict cortical excitability as measured by MEP amplitudes in Parkinson's disease patients. However, we found that higher MEPs were related to increased STN beta (rather than decreased STN beta as per our hypothesis). This could be due to coupling of STN beta to M1-gamma oscillations, which could influence motor cortical excitability.

Disclosures: K. Udupa: None. **A. Ghahremani:** None. **J.R. Wessel:** None. **U. Saha:** None. **T. Hoque:** None. **A.R. Aron:** None. **R. Chen:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.18/U11

Topic: E.03. Basal Ganglia

Support: KFO-247

Title: Modulation of subthalamic γ oscillations by movement parameters in Parkinson‘s disease

Authors: *R. LOFREDI[1], A. BOCK[1], W.-J. NEUMANN[1], J. HÜBL[1], S. SIEGERT[1], G.-H. SCHNEIDER[1], J. KRAUSS[2], A. KÜHN[1];
[1]Neurol., Klinik Für Neurologie - Charité Campus Virchow-Kli, Berlin, Germany; [2]Klinik für Neurochirurgie, Medizinische Hochschule Hannover, Berlin, Germany

Abstract: Question: Parkinson‘s disease (PD) is a common medical condition leading to slowness of movement. Recently, pathologically enhanced synchronized oscillations in the basal ganglia have been proposed as a pathophysiological hallmark of PD. Specifically, enhanced synchronization in the β band (13-30 Hz) has been associated with bradykinesia and rigidity in PD. Synchronization in the γ band (40-90 Hz) and of high frequency oscillations (HFO, >100 Hz) are more prominent ON medication in PD and considered prokinetic. Pallidal γ band oscillations have been shown correlated to movement amplitude and velocity. Here we investigated if γ oscillations in the human subthalamic nucleus (STN) are related to scaling of movement.
Methods: 16 PD patients (4 female, mean age 58.4 years +/-2.8 SEM) undergoing deep brain stimulation in the STN were included in the study. Bipolar local field potentials from 3 adjacent contact pairs were recorded while patients were on their usual dopaminergic medication. Patients performed forearm pronation movements of three amplitudes (‘small’, ‘medium’, ‘large’). Signals were taken into the frequency domain using wavelet analysis, averaged and normalized to a pre-trial baseline period. Normalized time frequency plots were averaged across hemispheres and patients. Statistical analysis was conducted using non-parametric Wilcoxon-sign rank tests. Averaged β (13-30 Hz), γ (40-90 Hz) and HFO (100-400 Hz) were compared across hemispheres, movement amplitude and velocity and correlated with the clinical symptom severity as measured by the UPDRS-III.
Results: Movement aligned analysis revealed a significant desynchronization in the β band and an increase of synchronization in the γ and HFO band ($p<.05$, FDR corrected) of the STN. Movement-related contralateral γ synchronization was modulated by movement amplitude with highest γ power in the ‘large’ movement condition within patients. Furthermore, increase of contralateral γ power correlated with averaged movement velocity (Spearman’s Rho=0.55, $p=0.018$) across all patients. Finally, mean power of contralateral γ oscillations correlated

negatively with UPDRS III (Spearman's Rho =-0.6, p=0.014).
Conclusions: Here we show that synchronization of subthalamic γ oscillations are related to movement velocity and amplitude in the ON medication condition supporting the notion that γ activity is a prokinetic feature in PD. Further analyses should reveal if γ synchronization is impeded by enhanced beta band activity OFF medication leading to slowness of movement.

Disclosures: **R. Lofredi:** None. **A. Bock:** None. **W. Neumann:** None. **J. Hübl:** None. **S. Siegert:** None. **G. Schneider:** None. **J. Krauss:** None. **A. Kühn:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.19/U12

Topic: E.03. Basal Ganglia

Support: Wellcome Trust Grant WT102849MA

UCL Grand Challenge

The GPU used for this research was donated by the NVIDIA Corporation.

Title: Cortico-basal ganglia connectivity in non-human primates: Implications for the therapeutic effect of STN stimulation in PD patients.

Authors: *A. MATIS[1], R. N. LEMON[1], D. C. ALEXANDER[2], A. KRASKOV[1];
[1]Sobell department of motor neuroscience and movement disorders, UCL Inst. of Neurol., London, United Kingdom; [2]Dept. of Computer Sci., Ctr. for Med. Image Computing, London, United Kingdom

Abstract: The connection between the basal ganglia and motor cortical areas is important in mediating normal response inhibition and has been proposed to play an important role in the pathology of Parkinson's disease (PD). Therefore, understanding cortico-basal ganglia connectivity and its alterations in disease is essential for understanding PD mechanisms and explaining and improving existing treatments such as deep brain stimulation (DBS). One plausible hypothesis as to how DBS of the subthalamic nucleus (STN) produces its therapeutic effect is by normalizing the activity of motor cortical neurons, which project directly to the STN and are antidromically activated by DBS (Gradinaru et al. 2009; Li et al. 2012). The main research question here is how the STN is connected to motor and other cortical areas across different primate species and how this connectivity changes with pathology and DBS treatment.We use probabilistic tractography to map cortical connections to the STN in four ex-

vivo macaque monkeys (data kindly provided by Evan Calabrese, Duke University, Calabrese et al. 2014) and compare these to results from conventional tracing studies (Haynes & Haber 2013).We found that STN had its strongest connectivity to limbic cortical areas (areas 11, 13, 14, 25, OPro, OPAll) and this connectivity was confined to medio-ventral regions of the STN. This area overlapped extensively with parts of the STN connected to cognitive cortical areas (areas 8-10, 44-46). We confirmed that the dorso-lateral part of the STN had its strongest connectivity to motor cortical areas (M1, SMA, PM) and fibers are likely to enter the STN from its dorso-lateral pole. These results speak in favour of two subdivisions of the STN: a lateral region connected to motor areas and a medio-ventral region to limbic and cognitive cortical areas.We further found that M1 input into STN was restricted to the dorso-lateral pole of STN whereas input from SMA was located more ventro-medial which is in line with tracing studies (Nambu et al.1996). The largest motor input to STN came from caudal dorsal premotor cortex (PMdc, 73.20 ± 54.44 out of 5000 streamlines) and rostral ventral premotor cortex (PMvr, 28.03 ± 8.77 out of 5000 streamlines) and was located medial to M1 input.To establish a connection between the activation of motor cortical neurons by STN DBS and its therapeutic effects in patients with PD, it is essential to understand the connectivity of the STN to these areas and the identity of these cortical neurons. This will have implications on the optimal location of DBS for specific motor deficits in PD patients and reduction of unwanted side effects.

Disclosures: **A. Matis:** None. **R.N. Lemon:** None. **D.C. Alexander:** None. **A. Kraskov:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.20/U13

Topic: E.03. Basal Ganglia

Support: NS045962

NS073994

Title: Phosphodiesterase 9 inhibition therapy for Parkinson’s disease

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Abstract: Striatal phosphodiesterases regulate selectively the cyclic nucleotides cAMP and/or cGMP that are involved in signaling pathways modulated by dopamine. Depending on the

isoenzyme substrate, selective inhibitors may impact differently L-dopa effects in Parkinson's disease (PD). Phosphodiesterase 9 (PDE9) regulates cGMP but not cAMP, thereby influencing particular dopamine receptor mechanisms in striatal projection neurons. In this study, we determined the effects of a selective PDE9 inhibitor in monotherapy and co-administration with L-dopa in the non-human primate (NHP) model of PD. Six macaques (*Macaca fascicularis*) were rendered severely parkinsonian by repeated MPTP administration, and maintained under regular treatment with L-dopa/carbidopa (Sinemet 100/25 mg). PDE9 inhibitor doses from 0.12 to 7.5 mg/kg (s.c.) were tested alone or in combination with either of two doses of L-dopa methyl ester plus benserazide (threshold and suboptimal doses). Standardized scales were used for blinded assessment of motor disability, levodopa-induced dyskinesias (LID), and other neurologic effects. Pharmacokinetics (PK) of the PDE9 inhibitor and L-dopa were studied and correlated with behavioral results. The PDE9 inhibitor (2.5 and 5 mg/kg) significantly potentiated the antiparkinsonian effect of L-dopa on acute tests in NHP. Motor disability scores were lower throughout the entire L-dopa response ($p < 0.01$), which was also longer demonstrating a clear prolongation of the "on" state ($p < 0.01$). PDE9 inhibition had significant effects on both threshold and suboptimal L-dopa responses. No direct PDE inhibitor effects on LID were observed. This agent had no significant motor effects on monotherapy. In all tests of the PDE9 inhibitor, no significant adverse reactions were observed. These results suggest that PDE9 inhibition may be a therapeutic strategy to extend and smooth responses to L-dopa facilitating the use of lower doses that also reduce abnormal motor responses.

Disclosures: S.M. Papa: None. **A. Singh:** None. **G. Masilamoni:** None. **X. Tang:** None. **L. Leventhal:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.21/U14

Topic: E.03. Basal Ganglia

Support: NS073994

Title: Striatal overexpression of ΔFosB leads to the development of dyskinesias in parkinsonian non-human primates without chronic levodopa exposure

Authors: *A. SINGH[1], L. F. POTTS[1], M. MARTINEZ[1], J. M. YOO[2], E. S. PARK[2], J. ZHANG[2], E. JUNN[2], M. M. MOURADIAN[2], S. M. PAPA[1,3];
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Abstract: The transcription factor ΔFosB has been involved in the pathogenesis of levodopa-induced dyskinesias (LID) due to the observed increase in its striatal expression in animal models and Parkinson's disease patients exposed to chronic levodopa treatment. However, the levels of striatal ΔFosB are already elevated after dopamine depletion, and the role of this transcription factor in the mechanisms of altered responses to levodopa is unclear. We studied the effect of striatal ΔFosB overexpression induced by a viral vector in parkinsonian non-human primates that were maintained naïve of levodopa treatment. rAAV-ΔFosB or GFP was infused in the putamen and caudate bilaterally in 8 cynomolgus monkeys with moderate-severe parkinsonism. The response to a levodopa (s.c.) dose was determined before the virus infusion as baseline, and 4 weeks after virus infusion the response to the same levodopa dose was tested weekly for 3 months. Animals did not receive daily levodopa treatment. In 4 animals, recording chambers were surgically implanted for single cell recordings to analyze the striatal projection neuron (SPN) activity during the weekly responses to levodopa. At the end of the study, the expression of ΔFosB or GFP in the striatum was determined in all animals. We found that the transgenic ΔFosB overexpression in the striatum caused rapid development of LID in all animals, and in some as early as in the first levodopa test post-AAV infusion. These results contrasted with the lack of LID in the animals receiving the control virus (rAAV-GFP), that were also tested with weekly levodopa injections just as were tested the rAAV-ΔFosB-infused animals. The electrophysiology data showed predominance of inversion of SPN firing frequency changes (bidirectional changes) during the response to levodopa, which characterizes the altered dopamine responses associated with the expression of LID. The increase in bidirectional responses correlated with the AAV-ΔFosB infusion and the appearance of LID. Conversely, a higher proportion of unidirectional responses were associated with the absence of LID in animals infused with the control virus. Immunoblotting analysis of protein extracts from striatal tissue obtained at the end of the study demonstrated consistent overexpression of ΔFosB (or GFP) across animals. These results demonstrate that the high striatal ΔFosB expression, independent of levodopa exposure, can lead to dyskinetic responses to levodopa, supporting a causal role of ΔFosB in LID development.

Disclosures: A. Singh: None. **L.F. Potts:** None. **M. Martinez:** None. **J.M. Yoo:** None. **E.S. Park:** None. **J. Zhang:** None. **E. Junn:** None. **M.M. Mouradian:** None. **S.M. Papa:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 415.22/U15

Topic: E.03. Basal Ganglia

Support: NIH R01-NS062876

NIH P50-NS071669

NIH OD P51-OD011132

Title: Effects of optogenetic activation of thalamostriatal terminals in monkeys.

Authors: *A. GALVAN[1], X. HU[2], Y. SMITH[1], T. WICHMANN[1];
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Abstract: The caudal intralaminar thalamic nuclei, i.e., the centromedian and parafascicular nucleus (CM/PF), provide massive thalamic input to the striatum, targeting spiny projection neurons and interneurons. Although CM/PF inputs are glutamatergic, we found previously (Nanda et al 2009) that electrical stimulation of the CM/PF evokes combinations of increases and decreases in firing in striatal neurons. To further investigate this issue, we studied the effects of selectively activating the terminals of the CM/PF-striatum connection, using an optogenetic approach.

Two monkeys received recording chambers for access to CM/PF and the caudate/putamen. We injected AAV5-hSyn-ChR2-EYFP or AAV5-hSyn-C1V1-EYFP in the CM/PF complex. Six 6 weeks later, we introduced optrodes (tungsten electrodes glued to 0.2 mm OD optical fibers) into the putamen or the caudate nucleus and activated opsin-positive terminals of the CM/PF-striatal projections with light pulses (>200 mW/mm2; single pulses, 500 ms duration), while recording the electrical activity of striatal neurons with standard extracellular recording methods.

We recorded the activity of 41 neurons in the caudate and 487 neurons in the putamen from the 2 monkeys. Approximately 40% of caudate and 20% of putamen neurons responded with significant decreases in firing during the light stimulation, while less than 10% of neurons showed increases in firing. The decreases in firing started, on average, 150 ms after the start of the light pulse, although some neurons responded with much shorter (< 10 ms) responses. Projection or interneurons (distinction based on unstimulated firing rates) responded to stimulation. The predominance of inhibitory effects of the optical activation of CM/PF terminals and the long latency of the striatal responses suggests that many of the effects were not monosynaptic, but that the transfected CM/PF terminals in the striatum exerted their effects by activating intrastriatal or other inhibitory pathways. Presumably, similar networks would also be engaged by the glutamatergic CM/PF inputs under physiologic conditions. We will conduct postmortem analyses of the brain tissue of these animals to study the distribution of the opsin-expressing CM/PF terminals in the striatum, as well as their cellular targets.

Disclosures: **A. Galvan:** None. **X. Hu:** None. **Y. Smith:** None. **T. Wichmann:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.23/U16

Topic: E.03. Basal Ganglia

Title: Altered functional connectivity associated with striatal dopamine depletion in Parkinson's disease

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Abstract: Introduction Cortico-basal ganglia circuit model, or firing rate model, successfully link striatal dopamine depletion and bradykinesia in Parkinson's disease (PD). However, the model provokes several conflicts with clinical observations. For example, the model is difficult to reconcile with the fact that lesions in the motor thalamus were found to relieves symptoms, rather than worsen bradykinesia in PD patients. **Methods** Seventeen PD patients and 16 healthy control subjects (HC) underwent [^{11}C]-CFT PET. Whole brain BOLD fMRI during hand movement and rest, T1 weighted image and field map data were also acquired with a 3T scanner. Analyses were performed using FMRIB Software Library (FSL) v5.0.7 tools. First, we set regions of interest (ROIs) on striatal area showing significant ^{11}C-CFT binding reduction in PD compared to HC. Then, we examined functional connectivity of the ROIs in PD and HC. **Results** The striatal ROIs demonstrated functional connectivity with the cortical motor areas, thalamus, internal gllobus pallidum (GPi) and substantia nigra (SN), in both PD and HC, though the strength of functional connectivity was decreased in PD compared with HC. In contrast, the strength of functional connectivity of the ROIs with the striatum, thalamus and subthalamic nucleus (STN) linearly increased in patients with severe bradykinesia, implicating a connection between the abnormal subcortical network and bradykinesia in PD. Meanwhile, the strength of functional connectivity of another network including the striatum, thalamus, and cerebellum correlated with the degree of tremor. **Discussion** Based on the present findings, we propose that striatal dopamine depletion decreased functional connectivity with the cortical motor areas, thalamus, GPi, and SN in PD. We also propose that emergence of abnormal functional network underlay motor symptoms in PD: increased functional connectivity among the striatum, thalamus and STN was associated with bradykinesia whereas enhanced connectivity among the striatum, thalamus and cerebellum was related to tremor.

Disclosures: A. Shima: None. **N. Sawamoto:** None. **R. Inano:** None. **H. Tabu:** None. **T. Okada:** None. **K. Togashi:** None. **R. Takahashi:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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MINECO grant SAF2012-039875-C02-01

grant from Jon Zarandona

Title: Morphological evidence supporting dopamine D1/D2 receptor heteromers in the striatum of the long-tailed macaque. Changes following dopaminergic manipulation.

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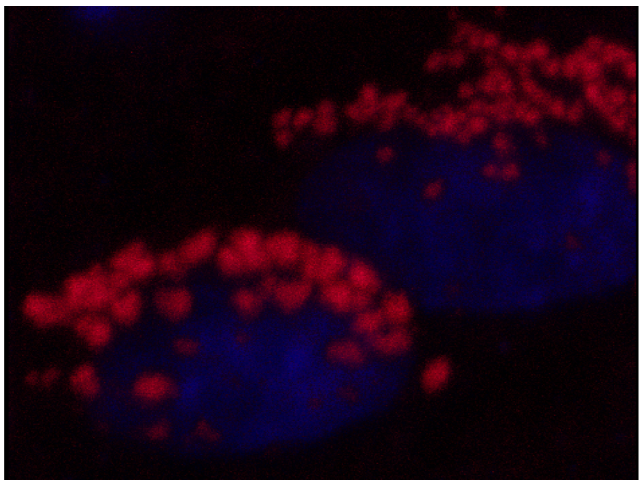
Abstract: Although it has long been widely accepted that dopamine receptors types D1 and D2 form GPCR heteromers in the striatum, the presence of D1/D2 receptor heteromers in the adult mammalian brain has been recently challenged. In an attempt to properly characterize the presence/absence of D1/D2 receptor heteromers, here we have used the *in situ* proximity ligation assay (PLA) in striatal sections comprising the caudate nucleus, the putamen and the core and shell territories of the nucleus accumbens. Experiments were carried out in control macaques as well as in MPTP-treated animals (with and without dyskinesia). Obtained data support the presence of D1/D2 receptor heteromers within all the striatal subdivisions, with the highest abundance in the accumbens shell. Dopamine depletion by MPTP resulted in an increase of D1/D2 density in caudate and putamen which was normalized by levodopa treatment. Two different sizes of heteromers were consistently found, thus suggesting that besides individual heteromers, D1/D2 receptor heteromers are sometimes organized in macromolecular complexes made of a number of D1/D2 heteromers. Furthermore, the PLA technique was combined with

different neuronal markers to properly characterize the identities of striatal neurons expressing D1/D2 heteromers. We have found that striatal projection neurons giving rise to either the direct or the indirect basal ganglia pathways expressed D1/D2 heteromers. Interestingly, macromolecular complexes of D1/D2 heteromers were only found within cholinergic interneurons. In summary, here we provide overwhelming morphological proof that D1 and D2 receptors form heteromeric complexes in the macaque striatum, thus representing a very appealing target for a number of brain diseases involving dopamine dysfunction



Disclosures: **J.L. Lanciego:** None. **A.J. Rico:** None. **I.G. Dopeso-Reyes:** None. **E. Martinez-Pinilla:** None. **D. Sucunza:** None. **D. Pignataro:** None. **E. Roda:** None. **D. Marin-Ramos:** None. **J.L. Labandeira-Garcia:** None. **S.R. George:** None. **R. Franco:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

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Topic: C.03. Parkinson’s Disease

Support: Chair in Neuroscience UAM-Fundación Tatiana Pérez de Guzmán el Bueno

CIBERNED

Fundación de Investigación HM Hospitales

MINECO SAF2015-67239-P

Title: Early loss of extra-striatal dopaminergic innervation in a progressive MPTP monkey model: a putative compensatory mechanism?

Authors: *I. TRIGO DAMAS[1], A. VIAN-LAINS[2], H. IWAMURO[3], J. BLESA[1], M. SANCHEZ-GONZALEZ[2], C. CAVADA[2], J. OBESO[1];
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Abstract: Traditionally, the major pathophysiological emphasis in Parkinson's Disease (PD) has resided in striatal dopamine (DA) deficit. However, the *substantia nigra pars compacta* (SNc) has projections to other basal ganglia (BG) nuclei; and the thalamus, brainstem and cortex are also innervated by DA axons. Earlier reports have also shown that DA loss in the subthalamic nucleus (STN) is already present at the onset of motor symtoms in both PD patients and in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of parkinsonism. Such DA loss in the STN would precede DA reduction in the external and internal segments of the globus pallidus (GPe/GPi). However, it is unclear how early in the evolution of pakinsonism extra-striatal DA depletion occurs. Moreover, the physiological effects of DA differ in the various BG nuclei. In order to examine the DA depletion in the extra-striatal BG nuclei, the length of DA axons was measured in the STN and GPe/GPi of 20 monkeys: a control group and MPTP-treated groups classified, according to their motor state, in asymptomatic, recovered and parkinsonian. The total length of axons immunoreactive for the DA transporter was estimated employing a hemispherical probe combined with unbiased fractionator sampling using StereoInvestigator® software (MicroBrightField Bioscience, Williston, VT, USA). The results show a decrease of ≈ 40% of axonal length and density in STN at early stages of nigro-striatal DA depletion, i.e. in the asymptomatic monkeys. The decrease is maintained through the progressive motor-impaired states. Moreover, we have examined the effects of Quinpirole (DA agonist) and Sulpiride (DA antagonist) microinjections within the STN and GPe in the motor behavior of parkinsonian monkeys. Briefly, the DA agonist in STN led to motor aggravation whereas the antagonist provoked modest improvement. Moreover, microinjections of the DA agonist in GPe, even if not so clear, also result in motor worsening of the animal, who also presented abnormal movements. We propose that DA denervation in the STN takes place early in the evolution of nigro-striatal degeneration and it may play an important compensatory role in the pre-symptomatic stage of PD. Accordingly, an early reduction of DA input would decrease neuronal excitability and delay the onset of abnormal firing patterns in the STN and other extra-striatal nuclei. This in turn would keep BG output within normal limits, thus delaying the onset of motor symptoms in PD.

Disclosures: I. Trigo Damas: None. **A. Vian-Lains:** None. **H. Iwamuro:** None. **J. Blesa:** None. **M. Sanchez-Gonzalez:** None. **C. Cavada:** None. **J. Obeso:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.26/V1

Topic: C.03. Parkinson's Disease

Support: Chair in Neuroscience UAM-Fundación Tatiana Pérez de Guzmán el Bueno

Fundación de Investigacion HM Hospitales

FIS PIE14/00034

SAF2012-40216

SAF2015-67239-P

Title: Dopaminergic neurons intrinsic to the striatum: a potential compensatory mechanism in Parkinson's Disease?

Authors: *J. BLESA[1,2], N. LOPEZ-GONZALEZ DEL REY[1], P. GARCIA-ESPARCIA[3,2], I. TRIGO-DAMAS[4,2], I. FERRER[3,2], C. CAVADA[5], J. OBESO[4,2];
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Abstract: The striatum harbors a population of neurons that express tyrosine hydroxylase (TH), an enzyme necessary for dopamine (DA) synthesis and a useful marker for DA neurons. The number of striatal TH-positive neurons (TH+) increases markedly in animal models of Parkinson's disease (PD). In humans, the presence and significance of TH+ neurons in the striatum of PD patients has been controversial. Likewise, the effect of L-dopa treatment and of DA striatal concentrations on the number of these neurons is unclear. Despite being relatively few in number, the TH+ neurons could be a local source of DA and thus, they may play a role in transmitting and distributing neuromodulatory signals in the DA denervated striatum of parkinsonism. The TH+ striatal neurons may act as a compensatory mechanism apt to be enhanced to alleviate or delay the clinical onset of PD. Therefore, it is crucial to understand well the population of TH+ neurons in order to comprehend more fully its pathophysiological role and its therapeutic potential. We have investigated the phenotype and fate of TH+ striatal neurons in non-human primates rendered parkinsonian by MPTP intoxication and in PD patients. Four control monkeys and 18 MPTP-intoxicated monkeys were studied. The latter included monkeys who were always asymptomatic, monkeys who recovered after showing mild parkinsonian signs, monkeys with stable parkinsonism, either moderate or severe, and L-Dopa treated parkinsonian

monkeys. Thus, unlike previous studies, we analyzed striatal TH+ neurons at different stages of the parkinsonian condition. Our results show that the numbers of TH+ neurons increase very early in the evolution of the nigro-striatal DA deficit, including the stage when no motor symptoms are present. They increase even further as the condition progresses to severe parkinsonism but their increment was completely abolished when parkinsonian monkeys were treated with L-Dopa. Similarly, PD patients examined in this study were also treated with L-Dopa to compensate for the loss of striatal DA. Accordingly, the striatum of PD patients was found to contain less TH+ neurons than that of controls. These data support the hypothesis that TH+ striatal neurons could serve as a compensatory mechanism for restoring striatal DA levels in the pre-symptomatic stages of experimental parkinsonism and of PD.

Disclosures: J. Blesa: None. **N. Lopez-Gonzalez del Rey:** None. **P. Garcia-Esparcia:** None. **I. Trigo-Damas:** None. **I. Ferrer:** None. **C. Cavada:** None. **J. Obeso:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.27/V2

Topic: C.03. Parkinson’s Disease

Support: CIHR (#MOP-115008)

Title: Dopamine and serotonin hyperinnervation of the globus pallidus in parkinsonian monkeys

Authors: *D. GAGNON[1], L. EID[1], C. WHISSEL[1], A. BRAUN[1], T. DI PAOLO[2], M. PARENT[1];
[1]CR-IUSMQ, Quebec, QC, Canada; [2]CR-CHUQ, Quebec, QC, Canada

Abstract: The primate pallidum is divided into an internal (GPi) and an external (GPe) segment, both receiving a highly heterogeneous serotonin (5-HT) and dopamine (DA) innervation. The main purpose of this light and electron microscopic study was to characterize neuroadaptive changes of 5-HT and DA axons in the GPi and GPe of a non-human primate model of Parkinson’s disease (PD). Eight cynomolgus monkeys (*Macaca fascicularis*) were used: 4 were rendered parkinsonian by systemic injections of MPTP and 4 served as controls. The state of 5-HT and DA pallidal innervation was evaluated by means of immunohistochemistry with antibody raised against the 5-HT transporter (SERT) and tyrosine hydroxylase (TH). The densities of immunolabeled axons and axon varicosities were estimated using an unbiased stereological approach. Overall, the GPi of macaque monkeys is more densely innervated by 5-HT than the GPe ($0.43 \pm 0.05 \times 10^6$ SERT+ axon varicosities/mm^3 vs. 0.32 ± 0.03). Intoxication

with MPTP induces a two-fold increase in the density of SERT+ axon varicosities in both pallidal segments ($0.75 \pm 0.07 \times 10^6$ SERT+ axon varicosities/mm^3 in the GPi and 0.61 ± 0.05 in the GPe). This change is accompanied by an increase in SERT+ axonal length in the pallidum. Ultrastructural features and synaptic incidence of SERT+ axon varicosities are similar between MPTP and control monkeys, in both pallidal segments. Our preliminary results of TH innervation indicate that the primate pallidum is less densely innervated by DA than by 5-HT axons with $0.18 \pm 0.09 \times 10^6$ TH+ axon varicosities/mm^3 in the GPi and 0.14 ± 0.01 in the GPe. A significant increase of the TH pallidal innervation is noted following MPTP administration, and this augmentation is more pronounced in the GPi with $0.59 \pm 0.10 \times 10^6$ TH+ axon varicosities/mm^3. An increase in TH+ axonal length is also noted in the GPi of MPTP monkeys. In contrast to the GPi, the GPe of parkinsonian monkeys shows a significant numerical decrease of the large and non-varicose TH+ axons. Altogether, our data reveal that both the DA and the 5-HT neuronal systems that innervate the primate pallidum are highly plastic and able to reorganize themselves in a significant manner in MPTP-intoxicated monkeys. In contrast to the massive degeneration of the nigrostriatal DA system that characterizes the PD state, the DA projection to the GPi, the major output structure of the basal ganglia, appears to be preserved and even increased in PD monkeys. We hypothesize that, based on their remarkable resistance and flexibility in face of a major neurotoxic insult, the DA and 5-HT pallidal inputs play a significant role in the expression of motor and non-motor symptoms of PD and of L-Dopa-induced dyskinesia.

Disclosures: D. Gagnon: None. **L. Eid:** None. **C. Whissel:** None. **A. Braun:** None. **T. Di Paolo:** None. **M. Parent:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.28/V3

Topic: C.03. Parkinson’s Disease

Support: Parkinson Society Canada

Pacific Parkinson's Research Institute

Title: Aerobic exercise can induce dopamine release in Parkinson's disease: [^{11}C]Raclopride PET study

Authors: *M. A. SACHELI[1], B. LAKHANI[2], J. L. NEVA[2], D. K. MURRAY[3], N. VAFAI[4], J. MCKENZIE[1], N. NEILSON[1], K. DINELLE[4], I. S. KLYUZHIN[4], L. A. BOYD[2], V. SOSSI[4], A.

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[1]Djavad Mowafaghian Ctr. for Brain Health, Univ. of British Columbia, Pacific Parkinson's Res. Ctr., Vancouver, BC, Canada; [2]Dept. of Physical Therapy, Fac. of Medicine, Djavad Mowafaghian Ctr. for Brain Health, Univ. of British Columbia, Vancouver, BC, Canada; [3]Fac. of Medicine, Univ. of British Columbia, Vancouver, BC, Canada; [4]Dept. of Physics and Astronomy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Objective: It is suggested that exercise may improve motor function in Parkinson's disease (PD) through increased dopamine (DA) release. The purpose of this study was to investigate differences in DA release induced by exercise or repetitive transcranial magnetic stimulation (rTMS) in participants with PD.
Methods: 28 PD participants were allocated into habitual exercisers (n=8) or sedentary (n=20) based on self-reported exercise frequency and verified by maximal oxygen consumption (VO_2 max). To examine DA release, participants underwent two [^{11}C]Raclopride (RAC) PET scans separated by either (a) 30min of cycling at a target intensity of 60% VO_2 reserve (0.6 x (VO_2 max - VO_2 rest) + VO_2 rest) (habitual n=8, sedentary n=9), or (b) rTMS over the primary motor cortex of the least affected hemisphere (sedentary n=11). Change in binding potential (ΔBP) (post stimulation - baseline scan=DA release) was examined in 8 regions of interest (ROIs) in the caudate and putamen. A texture and shape analysis will also be conducted to determine the change in spatial distribution of RAC.
Results: A group by hemisphere interaction ($F_{(2, 23)}$=8.31, p<.01) for ΔBP_{ND} was found in caudate. Fisher's LSD post-hoc test revealed the exercise stimulus to have significantly greater DA release in PD habitual exercisers compared to sedentary PD subjects ($p<0.01$ in both hemispheres), as well as compared to rTMS induced DA release ($p<0.01$ in both hemispheres)(fig. 1). There were no differences observed in the putamen in habitual exercisers or sedentary subjects for either stimuli.
Conclusion: These findings suggest that aerobic exercise is a suitable stimulus to elicit DA in caudate of habitual exercisers. Additionally, rTMS did not elicit consistent DA release in sedentary PD subjects. Further analysis on rTMS in habitual exercisers needs to be conducted.

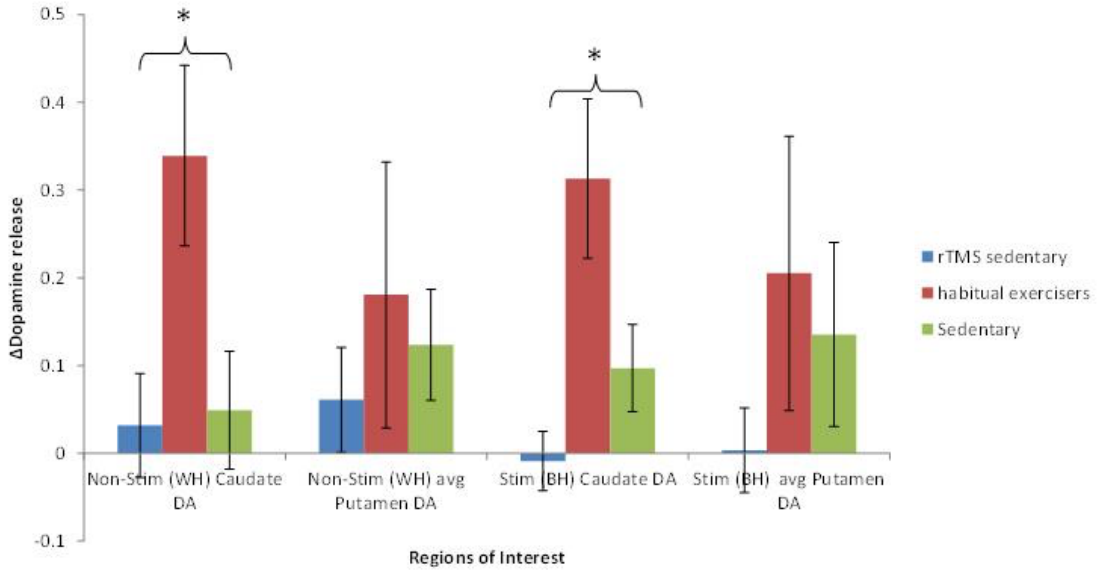

Figure 1. Change in binding potential with [^{11}C]Raclopride in Parkinson's disease subjects who are sedentary or habitual exercisers. Regions of interest (ROIs) include the caudate and average putamen (mean of anterior, middle and posterior ROIs). Δ Dopamine release, baseline binding potential – Post stimulus. Exercise stimulus: 30 minutes of aerobic exercise (cycling on a bicycle at 60 rpm) at 60% of maximal oxygen consumption (VO_2) reserve in PD habitual exercisers and sedentary PD subjects. Repetitive transcranial magnetic stimulation, rTMS: 4x150 stimulations at 90% resting motor threshold at 10Hz, 10 min rest between blocks in sedentary PD subjects. WH, worse hemisphere contralateral to the more affected side of the body (non-stimulated hemisphere); BH, better hemisphere (stimulated hemisphere). Error bars denote standard error of the mean. * $p<0.01$

Disclosures: **M.A. Sacheli:** None. **B. Lakhani:** None. **J.L. Neva:** None. **D.K. Murray:** None. **N. Vafai:** None. **J. McKenzie:** None. **N. Neilson:** None. **K. Dinelle:** None. **I.S. Klyuzhin:** None. **L.A. Boyd:** None. **V. Sossi:** None. **A.J. Stoessl:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 415.29/V4

Topic: C.03. Parkinson's Disease

Support: KAKENHI 15K08707

KAKENHI 15K12779

Brain/MINDS from AMED

Title: Plastic resting-state networks in MPTP-treated monkeys

Authors: *J. A. AUTIO[1], N. TANKI[1], T. OSE[1], J. TAKAHASHI[2], T. HAYASHI[1];
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Abstract: Introduction: Synchronized network activity is thought to play a central role in motor coordination, yet their role in pathophysiology of Parkinson Disease remains unclear. Converging electrophysiological and fMRI studies show anomalous synchronizations of cortico-basal ganglia circuits, however, whether these correspond with loss of dopaminergic neurons and Parkinsonian motor symptoms remain inconclusive. Here, we investigated neuronal synchronization in Parkinsonian animal model using fMRI and PET in anesthetized monkeys.
Methods: Study consisted a total of 14 monkeys: 6 were treated with repeated and 2 with a single-dose injection of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). MRI experiments were performed using 3 T scanner in combination with a custom-made 16-channel coil. Resting-state scans (TR 760 ms, $1.5 \times 1.5 \times 1.8$ mm^3) comprised 150 minutes per animal. Data was analyzed using temporal function mode (TFM), as described in Smith (2012, PNAS). Animals exposed to a single-dose MPTP injection, dopamine function was repeatedly assessed using dopamine transporter ligand, ^{11}C-PE21, and microPET.
Results: We found 6 TFMs in monkey brain, exhibiting similarity to the ones reported in humans (Smith, 2012). Animals with repeated MPTP-injection showed Parkinsonian motor symptoms and an increase in the sensorimotor TFM ($p < 0.05$, TFCE-corrected), particularly in sensorimotor and premotor areas and striatum as compared with non-MPTP treated animals (N=6). While a single-dose MPTP-injection in the unilateral carotid artery initially induced motor dysfunction and suppressed the binding of dopamine transporter, the sensorimotor TFM increased in the ipsilateral striatum and bilateral sensorimotor area. Strikingly, regardless of persistent ipsilateral dopaminergic loss after 1 month of MPTP injection, normalization of Parkinsonian motor symptoms coincided with network normalization.
Conclusions: Our results show that while partial dopaminergic dysfunction initiates sensorimotor network anomaly, network re-synchronization and behavioral recovery may occur despite persistent impairment of dopaminergic function. These results add additional weight to the idea that large-scale brain synchronization is essential for motor coordination and suggest that Parkinson Disease therapy may benefit from targeting functional network normalization.

Disclosures: J.A. Autio: None. **N. Tanki:** None. **T. Ose:** None. **J. Takahashi:** None. **T. Hayashi:** None.

Poster

415. Human and Non-Human Primate Therapies in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: R01-NS054976

P50-NS071669

OD P51-OD011132

Title: Phase amplitude coupling of electrocorticogram signals in a progressive model of primate parkinsonism

Authors: *A. DEVERGNAS[1,2,3], M. CAIOLA[4], D. PITTARD[1,3], T. WICHMANN[1,2,3]; [1]neurosciences, Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [2]Dept. of Neurology, Emory university, Atlanta, GA; [3]Udall Ctr. of Excellence in Parkinson's Dis. Reseach, EMory Univ., Atlanta, GA; [4]Dept. of Mathematical Sci., Rensselaer Polytechnic Institute, NY

Abstract: The degeneration of the nigrostriatal dopaminergic system in Parkinson's disease (PD) triggers pathologic oscillatory patterns in single cell and local field potential activities throughout the entire basal ganglia-thalamocortical network. As part of these changes, recent studies have described that the coupling of the phase of beta band oscillations and the amplitude of gamma band oscillations (50-200 Hz) of electrocorticogram signals from the primary motor cortex (M1) is stronger in PD patients than in patients with craniocervical dystonia or individuals without a movement disorder. The high level of phase-amplitude coupling (PAC) in parkinsonian patients was reduced by therapeutic deep brain stimulation. However, concomitant or past exposure to dopaminergic and other drug therapies may have affected these results. To clarify the relationship between cortical PAC and the motor impairment, we analyzed the relationship between the appearance of motor disabilities and the changes in PAC in electrocorticogram signals from M1 and the supplementary motor area (SMA) in two monkeys that underwent weekly injections of dopaminergic neurotoxin MPTP, rendering them progressively parkinsonian. We also examined the effect of antiparkinsonian levodopa treatment or therapeutic STN high frequency stimulation on cortical PAC in the fully parkinsonian state. We found that the development of parkinsonism was associated with increased coupling between the phase of alpha band oscillations (4-10 Hz) and the amplitude of high gamma band oscillations (50-150 Hz) in M1 and SMA. The changes were not detectable in the mildly parkinsonian state, but reached significance when the animals were more severely impaired. The increased alpha-gamma band PAC was significantly reduced after levodopa treatment or therapeutic STN stimulation. The PAC values were not correlated with the oscillatory power

found in the alpha or high gamma band. These results confirm that abnormal PAC is present in electrocorticogram signals in the advanced parkinsonian state. However, cortical PAC does not seem to be present in more mildly affected animals, suggesting that the PAC may not be essential for the development of parkinsonism, and that it may not be a sensitive biomarker for (early) PD.

Disclosures: **A. Devergnas:** None. **M. Caiola:** None. **D. Pittard:** None. **T. Wichmann:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

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Program#/Poster#: 416.01/V6

Topic: C.03. Parkinson's Disease

Support: NIH grant 1P01 NS059806

NINDS T32 NS086749

Title: GPx4 protects against oxidative stress-induced deficits in mitochondrial import: Implications for Parkinson's Disease

Authors: ***C. W. BARRETT**, P. J. BARRETT, A. D. MORTIMER, C. T. CHU, J. T. GREENAMYRE, T. G. HASTINGS, 15260;
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease. The hallmarks of PD include oxidative stress and mitochondrial dysfunction, both of which can be reduced with proper mitochondrial antioxidant systems. We have observed that a mitochondrial antioxidant protein is decreased in dopaminergic neurons in PD. The phospholipid hydroperoxide glutathione peroxidase (GPx4) is a potent redox active selenoprotein which is protective against ROS and membrane lipid peroxidation. GPx4 knockout is embryonic lethal and its inducible knockout in adult mice results in neurodegeneration, demonstrating the importance of GPx4 to development and survival. Some of the most essential machinery to mitochondrial health is intricately associated with the inner and outer mitochondrial membranes. For example, the mitochondrial import machinery, pivotal for uptake of 99% of mitochondrial proteins, is dependent on proper incorporation of cardiolipin into the inner mitochondrial membrane and maintenance of membrane potential. Of note, overexpression of mitochondrial GPx4 reverses a mitochondrial import defect in a mouse diabetic heart model suggesting that GPx4 can protect mitochondrial protein import. As GPx4 is pivotal to the protection of the

mitochondrial membrane from lipid peroxidation and is known to interact with cardiolipin, we hypothesized that *GPx4 would protect against oxidative damage-driven deficits in mitochondrial protein import*. In support of this hypothesis, GPx4 knockdown at 42% of endogenous levels in a dopaminergic cell line results in a 77% decrease in import of mitochondrially targeted GFP (N=3, $P<0.01$) and overexpression of mitochondrial GPx4 results in a 63% increase in import into isolated brain mitochondria (N=9, $P<0.05$). Treatment of isolated brain mitochondria with increasing concentrations of the oxidative stressors iron-ascorbate or tertiary-butyl hydroperoxide resulted in significant decreases in import of a radioactive mitochondrially targeted protein, and mitochondria isolated from GPx4 overexpressing mice (GPx4 Tg^{+}) demonstrated rescue of these import deficits. The mechanism for import protection may be due to decreased oxidative damage within the membrane and membrane-associated proteins in response to GPx4 overexpression. Indicative of increased functional import, there is a 3-fold increase in the proximity, assessed by proximity ligation assay, of mitochondrial import proteins Tom20 and Tom22 in mice overexpressing GPx4. These data suggest that selenium supplementation and/or upregulation of GPx4 expression protect mitochondrial function and may be beneficial in PD patients.

Disclosures: **C.W. Barrett:** None. **P.J. Barrett:** None. **A.D. Mortimer:** None. **C.T. Chu:** None. **J.T. Greenamyre:** None. **T.G. Hastings:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.02/V7

Topic: C.03. Parkinson’s Disease

Support: DSF Charitable Foundation

Blechman Foundation

Consolidated Anti-Aging Foundation

American Parkinson Disease Association

NIH Grant NS095387

Title: Alpha synuclein binds tom20 and impairs mitochondrial protein import in parkinson’s disease

Authors: *P. BARRETT[1], R. DIMAIO[1], E. HOFFMAN[1], C. BARRETT[1], A. ZHARIKOV[1], C. CHU[2], E. BURTON[1], T. HASTINGS[1], J. GREENAMYRE[1];
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Abstract: α-synuclein and mitochondrial impairment have both been implicated in the pathogenesis of Parkinson's disease (PD). Mitochondrial dysfunction leads to accumulation, oligomerization and aggregation of synuclein. Conversely, overexpression of synuclein leads to mitochondrial impairment by unknown mechanisms. We report that certain modified forms of synuclein interact specifically with the TOM20 receptor for mitochondrial protein import. Using a proximity ligation assay, we show that this interaction is blocked competitively by expression of a mitochondrial targeting sequence (MTS) peptide, the normal ligand of TOM20. Using mitochondrial protein import assays, we demonstrate that this interaction is sufficient to block MTS-mediated protein import. To investigate the roles of post-translational modifications of synuclein in this effect, we added monomeric synuclein, oligomeric WT synuclein, dopamine (DA)-modified synuclein, nitrated synuclein and an S129 phosphomimetic synuclein mutant to cells. Monomeric and nitrated synuclein had little effect, while oligomeric, DA-modified and phosphomimetic synuclein potently inhibited import. Inhibition of mitochondrially targeted proteins resulted in a decrease in mitochondrial respiration and complex I activity and an increase in oxidative stress. Using fluorescence spectroscopy, we measured direct binding of synuclein to the cytoplasmic domain of TOM20 and found specific binding ($K_d < 5\mu M$) of oligomeric, DA-modified and phosphomimetic synuclein, but not monomeric or nitrated species. Blinded postmortem assessment of human substantia nigra specimens revealed a strong association between synuclein and TOM20 in PD cases compared to controls, with an associated loss of a mitochondrially imported complex I subunit. To determine the extent to which endogenous synuclein contributes to the toxicity of mitochondrial impairment *in vivo*, we used a viral vector to knock down synuclein in substantia nigra and exposed rats to rotenone. Synuclein knockdown prevented the synuclein-TOM20 interaction and preserved levels of mitochondrially imported protein. Lastly, we showed that overexpression of TOM20 preserves mitochondrial protein import in the presence of oligomeric, DA-modified, and phosphomimetic synuclein. We conclude that post-translationally modified alpha-synuclein specifically interacts with the TOM20 receptor to inhibit mitochondrial protein import, resulting in senescent mitochondria that produce less energy and more reactive oxygen species. Thus, we have uncovered a novel pathogenic mechanism in PD that may provide a new target for therapeutic intervention.

Disclosures: P. Barrett: None. **R. DiMaio:** None. **E. Hoffman:** None. **C. Barrett:** None. **A. Zharikov:** None. **C. Chu:** None. **E. Burton:** None. **T. Hastings:** None. **J. Greenamyre:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.03/V8

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS08447

NIH Grant NS076160

NIH Grant NS092093

Title: Pathogenic alpha-synuclein mediates Parkinson's disease-related synaptic dysfunction and cognitive decline

Authors: *C. GALLARDO[1], A. COVELO[2], B. SINGH[3], H. A. MARTELL-MARTINEZ[2], M. A. BENNEYWORTH[4], A. ARAQUE`[2], M. K. LEE[5];
[1]Univ. of Minnesota Syst., Minneapolis, MN; [2]Neuroscience, Inst. for Translational Neurosci., [3]Neurosci., [4]Neuroscience, Inst. for Translational Neuroscience, Mouse Behavior Core, [5]Neuroscience, Inst. for Translational Neuroscience, Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Parkinson's disease (PD) is a common, progressive, neurodegenerative disorder that currently afflicts almost 2% of the population over 65 years of age. This number is expected to double by 2030. While motor dysfunction, resulting from the loss of dopaminergic (DAergic) neurons in the substantia nigra, is a cardinal feature of PD, the majority of individuals with PD experience non-motor symptoms with cognitive dysfunction being one of the most prominent and debilitating non-motor symptom. Such co-morbidities in PD are unrelated to the loss of DAergic neurons since they are not resolved by DAergic therapy. The current view is that α-synuclein (αS) pathology in the cortex and hippocampus may be responsible for the dementia in PD as well as in Dementia with Lewy Bodies (DLB), a disease highly related to PD. Together, PD and DLB comprise the second leading cause of dementia behind Alzheimer's disease. Thus, overcoming the limitations of current treatments and therapies to address this clinical burden relies on a better understanding of disease pathophysiology.
To elucidate the mechanisms underlying αS-mediated cognitive dysfunction in PD, we utilized transgenic (Tg) mice overexpressing wild-type (WT) human αS or αS harboring familial PD-linked mutations (A53T or A30P mutation). Cohorts of Tg mice and their non-Tg littermates were subjected to a variety of behavioral, electrophysiological and biochemical assays at defined ages up to one year old. We first demonstrate, using Barnes Maze and Fear Conditioning, that mice expressing the A53T mutant human αS (termed G2-3) display progressive memory impairment that precedes the onset of motor deficits. Consistent with the lack of overt pathology,

Tg mice expressing WT and A30P mutant human αS do not show memory impairment at 12 months of age. We explored whether the memory deficits could be linked to αS-induced synaptic deficits in hippocampal slices. Our analysis reveal that all three human αS Tg lines exhibit reduced number of spontaneous synaptic activity, consistent with αS being a general regulator of presynaptic function. Significantly, slices from A53T mutant αS Tg mice also showed reduced amplitude of spontaneous synaptic activity and reduced AMPA:NMDA ratio, suggesting a postsynaptic pathophysiology unique to this mutation. Taken together, our results provide a potential novel mechanism for αS-dependent regulation of synaptic activity where pathologic αS, such as the A53T mutant αS induces postsynaptic changes and cognitive impairment *in vivo*.
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Disclosures: C. Gallardo: None. **A. Covelo:** None. **B. Singh:** None. **H.A. Martell-Martinez:** None. **M.A. Benneyworth:** None. **A. Araque`:** None. **M.K. Lee:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.04/V9

Topic: C.03. Parkinson’s Disease

Support: CONACyT Grant 233815

Title: Assessing the maximum activity of the caspase 3 and 9 in a exerimental model of parkinson's disease induced by mpp+ in rats.

Authors: *M. ISLAS[1], M. RUBIO-OSORNIO[2], I. SANTANDER-RODEA[3], S. ZAMUDIO[1], C. RIOS[3], A. DIAZ-RUIZ[3];
[1]Inst. Politècnico Nacional, México D.F., Mexico; [2]Lab. Exptl. de Enfermedades Neurodegenerativas, Inst. Nacional de Neurología y Neurocirugía, ‘‘Manuel Velasco Suarez’’, Mexico D.F., Mexico; [3]Dept. de Neuroquímica., Inst. Nacional de Neurología y Neurocirugía, ‘‘Manuel Velasco Suarez’’., Mexico, D.F., Mexico

Abstract: Introduction: Parkinson’s disease (PD) is the second most common neurodegenerative disorder around the world. It is characterized by the loss of dopaminergic neurons of the *substantia nigra pars compacta* and striatal dopamine deficiency. Several reports in the literature indicate that neuronal death in PD occurs as a result of oxidative damage, due to an imbalance of free radicals to antioxidant mechanisms. Under these conditions of free radicals' overproduction, programmed cell death (apoptosis) can be started. The dynamics of that apoptotic cell death is unknown. Thus, the aim of this work was to determine the time-course of

the caspase 3 and caspase 9 activities, as markers of apoptosis in a model of PD induced by MPP+ in rats. **Experimental procedures:** Male Wistar rats (250-280 g of body weight) were used throughout the study. MPP+ iodide dissolved in sterile saline (10μg/8μL) was intrastriatally microinjected, using an stereotaxic frame. We used the following stereotaxic coordinates: 0.5 mm anterior to bregma, -3.0 mm lateral to bregma and -4.5mm ventral to the dura in the right striatum. The animals were killed by decapitation at 24, 48, 72, 120 and 168 hours after MPP+-injection to establish the time-course for maximal activity of caspases 3 and 9 in the striatum. **Results** showed an increased activity of both caspase 9 (28.3 ± 7.4) and caspase 3 (176.3 ± 26.6) at 120 hours after MPP+, as the highest value of caspase activation after injection. **Discussion;** The findings of this study allowed us to identify the time course of caspase activation after MPP* to further characterize the kinetics of apoptosis, that in turn may help us to develop neuroprotective strategies in the model of Parkinson´s disease. CONACyT Grant 233815

Disclosures: **M. Islas:** None. **M. Rubio-Osornio:** None. **I. Santander-Rodea:** None. **S. Zamudio:** None. **C. Rios:** None. **A. Diaz-Ruiz:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.05/V10

Topic: G.05. Anxiety Disorders

Title: Chronic psychological stress promotes PINK1/parkin-mediated mitophagy in mouse amygdala

Authors: ***K. DUAN**[1], X. LIU[2], Z. LI[1];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Key Lab. of Med. Neurobio. and Pharmacol. Res. Ctr., Fudan Univ., Shanghai, China

Abstract: Mitophagy is a specialized autophagy pathway that mediates the removal of damaged mitochondria by lysosomes for mitochondrial quality control. Since mitochondria are vulnerable to stress, mitophagy under stress conditions may play an important role in the stress-related psychopathology. Here we report that exposure of mice to chronic psychological stress correlates closely with the development of mitochondrial dysfunction in the amygdala, which in turn recruits PINK1/parkin-mediated mitophagy. Our results showed that mitochondria manifest abnormalities at the molecular, structural and functional levels particularly in amygdala after exposure to chronic social defeat stress (CSDS) 30 days. The mtDNA copy number in amygdala is significantly decreased (50.99%) at CSDS 30 days, but unchanged at CSDS 10 days. The stress enhances parkin or LC3 translocation to mitochondria and mt-KeimaRed translocation to

lysosomes in basolateral amygdala (BLA). Moreover, the PINK1 and parkin protein expressions are increased in homogenate of amygdala tissue after CSDS (154.77% and 136.33%). Thus the mitochondrial dysfunction and subsequent mitophagy in amygdala are potentially involved in the psychological stress-related psychopathology.

Disclosures: K. Duan: None. **X. Liu:** None. **Z. Li:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.06/V11

Topic: C.03. Parkinson's Disease

Support: Canadian Institutes of Health Research

National Science and Engineering Research Council

Title: Paraquat and stress interactions as pertains to Parkinson disease motor deficits and co-morbid behaviors

Authors: *K. FARMER, C. RUDYK, Z. DWYER, J. MCNEILL, F. WAHBEH, N. PROWSE, S. HAYLEY;
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Abstract: The impact of psychological stressors on the progression of motor and non-motor disturbances observed in Parkinson's disease (PD) has previously received little attention. Animal models have demonstrated that chronic unpredictable stress models recapitulate core symptoms of anxiety and depression in addition to altering central inflammatory and neurochemical processes. These alterations may increase vulnerability in PD-relevant brain regions, increasing susceptibility to further insults. Interestingly, chronic stress has been found to exacerbate the neurochemical and behavioral disturbances induced by 6-hydroxydopamine (6-OHDA), however, whether the impact of the PD relevant pesticide paraquat is enhanced in the context of chronic stressor exposure has yet to be examined. In the current study we examined the effects of chronic stress on behavioral disturbances induced by sub-chronic paraquat administration. We found that paraquat exposed animals with a backdrop of chronic stress did not significantly alter motor behaviors compared to animals receiving paraquat alone, however, these animals did display reductions in sucrose preference earlier than animals who received paraquat alone. In addition, the combined treatment also resulted in cognitive and anxiety disturbances greater than those produced by stress of paraquat alone. Furthermore, post-mortem

analysis revealed that paraquat affects stress-sensitive brain regions as alterations in glucorticoid receptor expression in the hippocampus and plasma corticosterone levels were seen, an effect that was exacerbated when in the presence of a chronic stressor. These results suggest that chronic stress may interact with paraquat exposure to produce behavioral disturbances.

Disclosures: **K. Farmer:** None. **C. Rudyk:** None. **Z. Dwyer:** None. **J. McNeill:** None. **F. Wahbeh:** None. **N. Prowse:** None. **S. Hayley:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.07/V12

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01-NS082205

Title: GBA1 deficiency plays a role in the formation of physiological α-synuclein tetramer

Authors: ***S. KIM**, S. YUN, S. LEE, H. KO;
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Abstract: Accumulating evidence suggests that α-synuclein occurs physiologically as a helically folded tetramer that resists aggregation. However, the mechanisms underlying the regulation of formation of α-synuclein tetramer are still mostly unknown. Cellular membrane lipids are thought to play an important role in the regulation of α-synuclein tetramer formation. Since GBA1 deficiency contributes to the aggregation of α-synuclein and leads to changes in the neuronal glycolsphingolipids (GSLs) including gangliosides, we hypothesized that GBA1 deficiency may affect the process of the α-synuclein tetramer formation. Here, we show that accumulation of GSLs due to GBA1 deficiency decreases the α-synuclein tetramer and increases α-synuclein monomer in neurons carrying heterozygous L444P GBA1 mutation. Moreover, the α-synuclein tetramer is decreased in N370S GBA1-PD iPSC-derived human dopaminergic neurons, CRISPR-GBA1 knockout SH-SY5Y cells, and stable SH-SY5Y cell lines expressing L444P GBA1. Strikingly, treatment of miglustast to reduce GSLs accumulation and overexpression of GBA1 to augment GBA1 activity reverse the destabilization of α-synuclein tetramer formation and protects against α-synuclein preformed fibrils induced toxicity in those cells. Taken together these studies could provide new mechanistic insights into how GBA1 deficiency due to GBA1 mutations contributes Parkinson's disease (PD) and Dementia with Lewy Body (DLB) and lead to unique therapeutic opportunities for PD and DLB.

Disclosures: **S. Kim:** None. **S. Yun:** None. **S. Lee:** None. **H. Ko:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 416.08/V13

Topic: C.03. Parkinson's Disease

Support: Department of Science and Technology

Indian Institute of Technology Madras

Title: Effect of trans fatty acid on alpha synuclein channels and implications in Parkinson's Disease

Authors: ***S. SRIDHAR**, A. K. BERA;
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Abstract: Dietary *trans* fatty acids (TFAs) are known to increase the risk of heart attack. However, their role in neurodegenerative diseases is not clear. We studied the effect of elaidic acid, the most abundant TFA found in food, on channel forming properties of alpha synuclein (ASN). ASN has been implicated in Parkinson's disease. The oligomeric species of ASN has been shown to be the toxic species which spreads the neuronal damage. Human ASN oligomers readily formed channels in planar lipid bilayer (PLB), composed of diphytanoyl phosphatidyl choline (DPhPC) and oleic (*cis*) or elaidic (*trans*) acid. When elaidic acid was incorporated in PLB, ASN channels exhibited different properties. The channels showed frequent but brief closures and longer open times in membranes with TFA as compared to *cis* lipid containing membranes. Also, the single channel conductance was greater in channels in TFA containing membranes with respect to those in *cis* lipid membranes. We are currently investigating the ASN toxicity in cultured neuron, pretreated with TFA. In conclusion, having TFAs in neuronal membranes could potentially exacerbate the neurodegeneration that occurs in Parkinson's disease.

Disclosures: **S. Sridhar:** None. **A.K. Bera:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 416.09/V14

Topic: C.03. Parkinson's Disease

Support: NRF Grant

Title: Self-defense responses against 6-hydroxydopamine-induced nitrosative cell death in C6 glioma cells

Authors: H. MOON[1], C. LEE[2], G. PARK[1], *J.-H. JANG[2];
[1]Col. of Pharm., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [2]Dept. of Pharmacol., Keimyung University, Sch. of Med., Daegu, Korea, Republic of

Abstract: Parkinson's disease (PD) is a neurodegenerative disease, which is caused by degeneration of dopaminergic neurons in the substantia nigra. As an experimental cellular model of PD, 6-hydroxydopamine (6-OHDA), a representative dopaminergic neurotoxin is widely used to cause an oxidative cell death in neuronal cells. In the present study, we have investigated 6-OHDA-induced neuronal cell death focusing on the nitrosative stress and accompanying adaptive survival responses in C6 glioma cells. Treatment of C6 cells with 6-OHDA increased expression of inducible nitric oxide synthase and subsequent production of nitric oxide. Furthermore 6-OHDA treatment led to formation of peroxynitrite and nitrotyrosine. 6-OHDA-induced nitrosative stress ultimately caused apoptotic cell death as determined by decreased Bcl-2/Bax ratio, activation of c-Jun N-terminal kinase, and cleavage of caspase-3 and poly(ADP-ribose)polymerase, which were attenuated by peroxynitrite decomposition catalyst, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron(III). In addition, exposure of C6 cells to 6-OHDA resulted in an increased expression of heme oxygenase-1 (HO-1) and 6-OHDA-induced cytotoxicity was effectively suppressed by carbon monoxide which is known to be produced by HO-1, supporting the cytoprotective role of HO-1. To elucidate the molecular mechanism underlying 6-OHDA-mediated HO-1 induction, we have examined the possible involvement of NF-E2-related factor 2 (Nrf2), which plays an important role in the transcriptional regulation of phase II detoxifying and antioxidant enzymes. 6-OHDA treatment increased nuclear translocation and transcriptional activity of Nrf2, which seemed to be partly mediated by activation of upstream kinases such as Akt/protein kinase B (PKB). Taken together these findings suggest that HO-1 up-regulation via Nrf2 activation may mediate the cellular adaptive survival response to 6-OHDA-induced nitrosative cell death in C6 glioma cells.

Disclosures: H. Moon: None. **C. Lee:** None. **G. Park:** None. **J. Jang:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.10/V15

Topic: C.03. Parkinson's Disease

Support: Department of Veterans Affairs

NIH

Title: Glial cells and mast cells signaling pathways in neuroinflammation.

Authors: K. DURAISWAMY[1], R. THANGAVEL[1], S. ZAHEER[1], D. A. SANTILLAN[2], M. K. SANTILLAN[2], *M. M. THAKKAR[1], A. ZAHEER[1];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease and is due to the degeneration of dopaminergic neurons in the brain. Neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) metabolite 1-methyl-4-phenylpyridinium (MPP^+) activate glial cells and release several inflammatory mediators in PD. We have previously shown that brain protein glia maturation factor (GMF) and mast cells are implicated in neuroinflammation and neurodegeneration. Matrix metalloproteinases (MMPs) including MMP-3 is involved in neurodegeneration and mast cells are known to secrete MMP-3. Moreover, mast cell mediator mouse mast cell protease-6 (MMCP-6) and MMCP-7 could activate glial cells and neuronal cells in neurodegenerative diseases. However, how mast cells, glial cells and neurons interact with each other during neuroinflammatory response such as in PD is not yet clearly understood. In this study, we analyzed the effect of MPP^+, GMF or mouse mast cell proteases on mouse bone marrow-derived cultured mast cells (BMMCs) and mouse fetal brain-derived cultured brain cells on the release of inflammatory mediators MMP-3 and chemokine (C-C motif) ligand 2 (CCL2), and the expression of co-stimulatory molecules CD40 and CD40L (CD154) in glial cells and mast cells co-culture system. Inflammatory mediators were quantitated in the cell culture supernatant media by ELISA and the expression of co-stimulatory molecules were analyzed on the cultured cells by flow cytometry. MPP^+ and GMF induced the release of MMP-3 from BMMCs. MMCPs induced the release of MMP-3 as well as CCL2 from mouse astrocytes. Flow cytometry results showed increased CD40L expression by MPP^+ in BMMCs, in a co-culture system that consisted of both BMMCs and mouse glial cells. Similarly, GMF increased the expression of CD40 in astrocytes, in an astrocyte plus BMMCs co-culture system. In conclusion, our results indicate potential new therapeutic target for neurodegenerative diseases including PD.

Disclosures: **K. Duraiswamy:** None. **R. Thangavel:** None. **S. Zaheer:** None. **D.A. Santillan:** None. **M.K. Santillan:** None. **M.M. Thakkar:** None. **A. Zaheer:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.11/V16

Topic: C.03. Parkinson's Disease

Title: Selective Toll-like receptor inhibition occludes alpha-synuclein-induced pro-inflammatory signaling & cytokine release

Authors: ***A. M. HABAS**[1], S. NATALA[2], J. WONG[2], W. WRASIDLO[2], M. GILL[3], D. BONHAUS[3];
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Abstract: Synucleinopathies are a family of central nervous system (CNS) degenerative disorders characterized by deposition of insoluble alpha-synuclein-containing protein aggregates, neuronal and/or glial cell death and inflammation. Toll-like receptors (TLRs) play a critical role in innate immune system response to damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). DAMP/PAMP-mediated activation of TLR1/TLR2 or TLR2/TLR6 heterodimers triggers NFκB signaling resulting in pro-inflammatory cytokine release. Recently, secreted, high molecular weight (HMW) alpha-synuclein aggregates derived from over-expressing rodent neuroblastoma cells have been shown to stimulate pro-inflammatory signaling in CNS-derived microglia. Genetic- or antibody-mediated TLR2 inhibition occluded this signaling cascade, suggesting TLR2 inhibition may be a viable strategy to disrupting alpha-synuclein-mediated inflammation. Here using stable TLR2-expressing HEK and naïve monocyte/macrophage-derived heterologous cell lines, we confirm that HMW alpha-synuclein activates pro-inflammatory signaling. In addition, we show, using blocking antibodies, that specific inhibition of TLR1/TLR2 but not TLR2/TLR6 occludes alpha-synuclein-mediated pro-inflammatory signaling. Lastly, we show that TLR2-selective small molecules inhibit alpha-synuclein-mediated pro-inflammatory signaling, supporting TLR2-selective small molecule inhibition as a feasible therapeutic modality towards blocking alpha-synuclein-mediated CNS inflammation.
All authors are full-time employees of Neuropore Therapies, Inc.

Disclosures: **A.M. Habas:** None. **S. Natala:** None. **J. Wong:** None. **W. Wrasidlo:** None. **M. Gill:** None. **D. Bonhaus:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS073670

NIH Grant AGO48205

VA 101BX001844

Title: Glia maturation factor dependent activation of nod like receptor family pyrin domain containing 3 (NLRP3) inflammasome

Authors: H. JAVED[1], M. M. KHAN[1], R. THANGAVEL[2], K. DURAISAMY[2], S. ZAHEER[2], S. IYER[2], *A. ZAHEER[2];
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Abstract: Neuroinflammation is a major contributor to the pathogenesis of various neurodegenerative diseases including Parkinson's disease (PD). Mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exhibits microglial activation-induced inflammation, and nigrostriatal dopaminergic (DA) neuronal damage, and thus serve as an experimental model of PD. Nod-like receptor family pyrin domain containing 3 (NLRP3) is a key component of the macromolecular signaling complex called NLRP inflammasome that promotes caspase-1 dependent maturation of pro-IL-1β followed by cell death. Glia maturation factor (GMF) a proinflammatory protein that is up-regulated in the central nervous system in neurodegenerative conditions. In the present study, we have investigated the expression of NLRP3 and caspase-1 in the substantia nigra (SN) of human PD and non-PD brain by immunohistochemistry. NLRP3 and caspase-1 positive cells were found significantly increased in PD when compared to non-PD control brains. We further detected the expression of NLRP3 inflammasome in the glial cells following MPTP treatment in mice. GMF deficient mice ($GMF^{-/-}$) showed reduced expression of NLRP3 inflammsome in the glial cells of SN as compared to WT. Subsequently, we found IL-1β level was significantly increased in the mid brain of WT as compared to $GMF^{-/-}$ mice. We demonstrated the possibility that GMF-dependent activation of the NLRP3 inflammasome is involved in the maturation of IL-1β in MPTP-treated mice. Our overall data indicate that GMF deficiency is crucial for the survival of DA neurons, by modulating the NLRP3 inflammsome activation, in animal model of PD.

Disclosures: **H. Javed:** None. **M.M. Khan:** None. **R. Thangavel:** None. **K. Duraisamy:** None. **S. Zaheer:** None. **S. Iyer:** None. **A. Zaheer:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.13/V18

Topic: C.03. Parkinson's Disease

Support: Medical School and grants from Dirección General de Asuntos del Personal Académico (DGAPA IN222215), National Autonomous University of Mexico (UNAM).

Title: Effect of chronic systemic inflammation in the cytokines profile in central nervous system and its implication in suceptibility to damage by neurotoxin MPTP in mice

Authors: *P. UGALDE MUÑIZ[1], A. CHAVARRÍA KRAUSER[2];
[2]Neuroinmunología, [1]Hosp. Gen. De México, Ciudad De México, Mexico

Abstract: Inflammatory processes and activation of microglia are both important factors in the pathogenesis of Parkinson's disease (PD). Recent studies demonstrated that systemic inflammation and neuroinflammation are both present in the progression of the disease. Evidence suggests that the activation of the peripheral immune system exacerbates the brain inflammatory response, which may initiate or enhance neurodegenerative processes. Recent data suggest that activation of the innate immune system, such as evoked by a peripheral pathogen, stimulates the pathogenesis of PD by generating a transient pro-inflammatory environment that is likely to accelerate neurodegeneration. The purpose of this work is to determinate if chronic systemic inflammation increases the inflammatory response in central nervous system in mice, and if this chronic inflammatory state turns animals more susceptible to damage by the neurotoxin MPTP. CD1 mice were used testing two strategies with LPS: 5 mg/kg i.p. (single dose) or 100 μg/kg i.p. (chronic dose) twice a week for three months. Both single and chronic doses were followed by MPTP intoxication (5 mg/kg i.p. for five days). To discern between the mechanisms of inflammation transfer from the periphery to the brain and the neurodegenerative consequences, levels of TNFα, IL-6, IL-10, and TGF-β were evaluated by ELISA in plasma and brain. As well as mitochondrial viability in the brain was evaluated by the assay of MTT reduction. Our results showed that chronic administration of LPS (100 μg/kg) decreased mitochondrial viability in the brain and increased levels of IL-6, TNFα, and TGF-β in both plasma and brain. The single dose of LPS (5 mg/kg) decreased mitochondrial viability in the brain as well; however, no changes were observed in the cytokine profile in the brain or plasma. On the other hand, the chronic dose of LPS together with MPTP increased IL-6 and TNFα, but not IL-10 and TGF-β in both plasma

and brain when compared with the group administered only with MPTP. In contrast, the single dose of LPS with MPTP evoked a raise in TNFα levels only in the brain. Our data suggest that a chronic inflammatory environment may associate with the progression of PD. We proved that the administration of systemic LPS could evoke an increase in the pro-inflammatory citokine profile, but not of anti-inflammatory cytokines. We believe that this previous chronic pro-inflammatory state may cause a susceptibility to a second inflammatory stimulus, exacerbating the pro-inflammatory cytokine production and reducing the anti-inflammatory mechanisms, all of this together could result in the death of dopaminergic neurons.

Disclosures: P. Ugalde Muñiz: None. **A. Chavarría Krauser:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.14/W1

Topic: C.03. Parkinson’s Disease

Support: Council for Scientific and Industrial Research(CSIR)

Department of Science and Technology(DST), Govt. of India

Title: Differential gene expression profile between early and late stage disease model of Parkinson's disease

Authors: *A. VERMA, V. RAVINDRANATH;
Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: The characteristic death of dopaminergic neurons in substantia nigra pars compacta(SNpc) leads to Parkinson’s disease(PD), a debilitating movement disorder. Although several putative mechanisms have been implicated in neuronal degeneration in SNpc, the factors that trigger selective cell death of dopaminergic neurons remain elusive. We therefore aimed to investigate the changes in gene expression from the early to late stage of disease pathogenesis using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) administered to mice sub-cutaneously at 30mg/kg body weight as a model of PD. Gene expression profiles were examined using RNA seq from SNpc at the early stage, 24 hrs post single exposure to MPTP and late stage, that is post 14 days of daily MPTP treatment where SNpc has undergone substantial cell death. The raw reads were taken through quality check using FastQC and were trimmed and filtered using Trimmomatic. They were aligned to mouse reference genome using TopHat2 and counts were assigned to the genetic loci using HTSeq. The data was further analysed for differential

expression of genes and miRNAs using DESeq2 and for differential exon usage and expression using DEXSeq. In addition, gene set enrichment analysis was performed on the data from both stages of disease pathogenesis using GAGE. Interestingly, it was observed that the expression of Nurr1, an important regulator of dopaminergic phenotype is down-regulated in the early stage of PD-disease pathogenesis and the down-regulation persists in late stage of disease. Dopaminergic neuronal markers such as tyrosine hydroxylase(TH), dopamine transporter(DAT), vesicular monoamine transporter(VMAT) and dopamine receptor D2 showed decreased gene expression only in the late stage implying cell death at this stage. Our results indicate that several pathways ranging from those involved in protein translation, splicing, oxidative phosphorylation, tumor necrosis factor α(TNFα) signalling etc. are perturbed early in the disease process and may play a role in initiating neurodegeneration in SNpc. RNA seq provides a reliable high throughput method for discovering perturbation in global gene expression networks thus providing insights into pathogenic processes.

Disclosures: **A. Verma:** None. **V. Ravindranath:** None.

Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 416.15/W2

Topic: C.03. Parkinson’s Disease

Support: Department of Science and Technology, India

Council of Scientific and Industrial Research, India

Title: Diamide induces selective and progressive dopaminergic neurodegeneration, α-synuclein pathology and Parkinsonism in mice: A model of Parkinson’s disease

Authors: *A. RAY, A. VERMA, S. KUMAR, V. RAVINDRANATH;
Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Oxidative stress has been demonstrated to play a critical role in pathogenesis of Parkinson’s disease (PD). We have shown that oxidative modifications of thiols, resulting in disruption of thiol homeostasis can trigger degeneration of dopaminergic neurons in substantia nigra *pars compacta* (SNpc). To test for specificity of degeneration, we stereotaxically administered a single, unilateral dose of the thiol oxidant, diamide, into either mouse SN or motor cortex. While SN injected mice demonstrated locomotor deficits and neurodegeneration in SNpc, similar defects were not observed upon diamide administration into motor cortex

indicating that SNpc neurons are highly vulnerable to thiol oxidation mediated by diamide. Mice injected with single dose of diamide into SN showed hemiparkinsonian motor impairment, which was accompanied by significant time-dependent progressive loss of dopaminergic neurons in ipsilateral SNpc and their striatal fibers. Frank neurodegeneration was confirmed by increased Fluoro-Jade C staining and loss of NeuN positive neurons. Importantly, diamide injection led to α-synuclein aggregation in ipsilateral SNpc, a hallmark of PD pathology not often seen in animal models of PD. Increased punctate phospho-α-synuclein and ubiquitin staining in SNpc cells further supported pathological α-synuclein aggregation. We observed loss of glutathione, essential for maintaining thiol homeostasis, coupled with increased lipid peroxidation, suggestive of ongoing oxidative stress. Concomitantly, the ASK1-p38 MAPK death signaling pathway was activated in ipsilateral but not contralateral ventral midbrain through oxidation of Trx1 and dissociation of ASK1-Trx1 complex. Since diamide selectively disrupts thiol homeostasis, dopaminergic neurons appear to be vulnerable to such perturbations, such that even a single exposure to thiol oxidant results in long-lasting progressive degeneration. Understanding the impact of thiol perturbation in neurodegeneration, especially in early PD, will not only reveal novel regulatory mechanisms of cellular signaling, but may aid in developing disease-modifying strategies for PD as well.

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Poster

416. Mitochondria, Alpha-Synuclein, and Inflammation in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 416.16/W3

Topic: C.03. Parkinson’s Disease

Support: NIH Grant T32 NS007433

NIH Grant NS059806

NIH Grant T32 NS086749

Title: Dopaminergic degeneration following viral mediated dysregulation of dopamine: Implications for Parkinson’s disease

Authors: ***M. BUCHER**, C. W. BARRETT, T. G. HASTINGS;
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Abstract: Dopaminergic neuronal health is dependent upon the proper handling of dopamine (DA). Following synthesis in the cytosol, DA is quickly packaged into acidic vesicles via the

vesicular monoamine transporter 2 (VMAT2). Disruptions in the packaging of DA can lead to increased cytosolic DA oxidation, forming ROS and highly reactive DA quinone, which can attack and covalently modify protein cysteinyl residues leading to detrimental downstream consequences. Cytosolic DA oxidation has been proposed to contribute to the neurodegenerative process in Parkinson's disease (PD). Previously, it was shown that mice expressing only 5% of normal VMAT2 levels showed an age dependent loss of substantia nigra (SN) DA neurons, and increased DA oxidation (Caudle et al.,2007). More recently, Pifl et al.(2014) showed impaired VMAT2 function within the remaining dopaminergic terminals in PD patients, suggesting that a dysregulation of DA storage can contribute to the pathogenesis of the disease. To investigate the effects of reduced VMAT2 expression in an adult animal, an adeno-associated virus containing a plasmid coding for small-hairpin ribonucleic acid against VMAT2 was generated (AAV2-sh[VMAT2]). Rats were stereotactically injected unilaterally into the right SN with AAV2-sh[VMAT2]. Six weeks following the viral injection, SN coronal sections were immunostained for VMAT2, tyrosine hydroxylase (TH), MAP2 and DAPI. Stereological counting of TH-and MAP2-positive neurons showed a significant loss of SN DA neurons on the AAV2-sh[VMAT2] injected side (-38.74%) compared to the contralateral non-injected SN ($N=5$; $p<0.05$). Likewise, decreased density of striatal TH-immunoreactive terminals and the presence of dystrophic axons on the viral injected side also suggested neurodegeneration. In the remaining transduced TH-positive SN neurons, VMAT2 protein levels were significantly decreased, and there was an increase in insoluble α-synuclein. Consistent with the loss of DA neurons, rats injected unilaterally into SN with AAV2-sh[VMAT2] showed significant asymmetric motor deficits as determined by the postural instability test and the cylinder test. These results show that a substantial reduction in the expression of VMAT2 within dopaminergic neurons is sufficient to cause neurotoxicity, implicating proper VMAT2 functioning and DA sequestration in the maintenance of dopaminergic neuronal health.

Disclosures: **M. Bucher:** None. **C.W. Barrett:** None. **T.G. Hastings:** None.

Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

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Program#/Poster#: 417.01/W4

Topic: C.04. Movement Disorders

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Title: Impaired autophagy in Huntington´s disease impacts on global microRNA-levels through Argonaute-2 accumulation

Authors: *K. PIRCS[1], R. PETRI[1], S. MADSEN[1], P. L. BRATTÅS[1], M. MATUSIAK-BRÜCKNER[1], R. VUONO[2], N. DÉGLON[3], R. A. BARKER[2], J. JAKOBSSON[1];
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Abstract: Many neurodegenerative disorders, such as Huntington's disease, are characterised by the formation of protein aggregates in the brain. However, despite intense research it remains still unclear if and how protein aggregation is implicated in the neuronal cell death. In this project we investigate a potential link from protein aggregation to cell death via autophagy and microRNAs in models of Huntington's disease.
To confirm that aggregation of mHTT protein results in impaired autophagy in the brain, we generated AAV vectors expressing mHTT with 66 CAG repeats and performed injections in the striatum of adult mice. We found that expression of mHTT resulted in time-dependent aggregation of mHTT that was associated with reduced levels of DARPP32 a marker of medium spiny neurons decreased in HD brains. Animals injected with mHTT displayed an induced autophagy 10 days after injection that were reversed at 3 and 8 weeks when autophagy was impaired. We found significantly more p62 by time coupled with elevated LC3-II levels suggesting a later autophagic block with autophagic flux impairment. In line with this, we found that mRNA-levels of transcription factors that activate autophagy were up regulated in mice 10 days after injection but at 3 weeks we saw the opposite, all the enhancers were down regulated. We also found an interesting trend for genes involved in lysosomal biogenesis, autophagosome maturation, cargo recruitment and vesicle trafficking: the fold changes of the mHTT/wtHTT mRNA levels were reversed. In almost all cases mRNA levels that were up regulated by 10 days after autophagy activation were decreased after 3 weeks due to autophagy impairment and vice versa.
Furthermore, we investigated a potential link between impaired autophagy and miRNA biogenesis in the mHTT mice and found an increased AGO2 protein level. AGO2 plays a central role in RNA silencing processes and it has been previously shown that selective autophagy degrades AGO2 and regulates miRNA activity. Furthermore, we found that mHTT expression and high AGO2 levels in mouse striatal neurons alters global levels of neuronal miRNAs. Additionally, we also observed that overexpression of BECN1 reverses mHTT-associated phenotypes by partially preventing aggregation of mHTT, decreasing the number of p62 aggregates and also reducing AGO2 aggregate size and number.
Overall, our data shows that mHTT aggregates impair autophagy on a protein and on a transcriptional level. Furthermore, we have shown that mHTT impairs clearance of protein aggregates and also alters the function of cellular cascades such as miRNA biogenesis.

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Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 417.02/W5

Topic: C.04. Movement Disorders

Support: NIH Grant NS078008

Title: Respiration and Ca^{2+} handling by striatal mitochondria purified from brains of YAC128 mice, a model of Huntington's disease.

Authors: ***J. HAMILTON**[1], T. BRUSTOVETSKY[1], N. BRUSTOVETSKY[1,2];
[1]Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Stark Neurosci. Res. Inst., Indianapolis, IN

Abstract: Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by motor and cognitive abnormalities and extensive striatal degeneration. HD pathogenesis is linked to a mutation in the protein huntingtin (Htt), which is normally involved in developmental processes and trafficking. The mechanistic link between mutant huntingtin (mHtt), selective striatal vulnerability, and HD pathogenesis is unknown. Previously, it was proposed that mitochondrial dysfunction could contribute to HD pathogenesis. In our previous studies with mitochondria isolated from YAC128 mice that express full-length human mHtt and R6/2 mice that express the exon 1-encoded fragment of human mHtt, we found no evidence for functional deficits in mitochondria from HD mice compared with mitochondria from WT mice. However, in these studies we evaluated functions of mitochondria isolated from the whole brain. In the present study, we investigated the effect of mHtt on respiration and Ca^{2+} uptake capacity of mitochondria isolated from striata of YAC128 mice. We genotyped every mouse to confirm the presence of the transgene and analyzed isolated mitochondria by western blotting for the presence of mHtt. We evaluated respiratory activity in Percoll gradient-purified nonsynaptic and synaptic striatal mitochondria from 2 month old, early symptomatic YAC128 mice and age-matched WT mice. We used western blotting to evaluate the expression of nuclear-encoded mitochondrial proteins in striatal mitochondria: 39 kDa subunit of Complex I, 30 kDa subunit of Complex II, 70 kDa subunit of Complex II, Aconitase 2, manganese-dependent superoxide dismutase, alpha subunit of ATP synthase, Cyclophilin D, and subunit IV of cytochrome *c* oxidase. We also assessed the effect of mHtt on Ca^{2+} uptake capacity in nonsynaptic and

synaptic striatal mitochondria isolated from YAC128 mice. In our experiments, we found no difference in respiratory activity and Ca^{2+} uptake capacity between striatal mitochondria from YAC128 mice and WT animals. The lack of mitochondrial dysfunction was supported by comparable expression of nuclear-encoded mitochondrial proteins in striatal mitochondria from YAC128 and WT mice. Overall, our data do not support mHtt-mediated impairment of mitochondrial respiratory activity and Ca^{2+} handling in striatal mitochondria from YAC128 mice.

Disclosures: J. Hamilton: None. **T. Brustovetsky:** None. **N. Brustovetsky:** None.

Poster

417. Huntington's Disease Mechanisms II

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FEDER through "Programa Operacional Factores de Competitividade – COMPETE" and FCT (UID/NEU/04539/2013; PEst-C/SAU/LA0001/2013-2014)

Title: Redox changes and bioenergetic defects in striatum and cortex of pre-symptomatic and symptomatic Huntington disease mice

Authors: *A. C. REGO[1,2], C. MARANGA[2], I. L. FERREIRA[2], M. LAÇO[2], S. MOTA[2], L. NAIA[1,2], J. SERENO[3], A. ABRUNHOSA[4,3], M. HAYDEN[5], M. CASTELO-BRANCO[4,3]; [1]Fac. of Med. and CNC, Coimbra, Portugal; [2]CNC-Center for Neurosci. and Cell Biology, Univ. of Coimbra, Coimbra, Portugal; [3]ICNAS – Inst. for Nuclear Sci. Applied to Health, Univ. of Coimbra, Coimbra, Portugal; [4]IBILI - Inst. for Biomed. Imaging and Life Sciences, Fac. of Medicine, Univ. of Coimbra, Coimbra, Portugal; [5]Ctr. for Mol. Med. and Therapeutics, Child and Family Res. Institute, Dept. of Med. Genetics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington disease (HD) is a neurodegenerative disorder strongly affecting the striatum and the cerebral cortex. HD is caused by a CAG expansion in the *HTT* gene encoding for mutant huntingtin (mHTT), which affects mitochondrial function and redox regulation, leading to oxidative stress. In this study we assessed mitochondrial deregulation and modified redox changes in HD through *in vivo* PET brain studies for detection of oxidative stress using

radiolabeled Cu(II)-ATSM and *ex vivo* mitochondrial function and bioenergetic analysis of brain mitochondria isolated from pre-symptomatic (3 month-old, mo) and symptomatic stages (6-12mo) YAC128 transgenic mice *versus* age-matched wild-type mice. YAC128 mice at initial symptomatic stages exhibited an increase in Cu(II)-ATSM uptake in striatum and, at a lower extent, in hippocampus, but not in pre-frontal cortex. Because Cu(II)-ATSM uptake has been attributed to mitochondrial-associated oxidative stress, we analyzed the production of hydrogen peroxide (H_2O_2) in striatal and cortical mitochondria of YAC128 mice. Interestingly, 3mo YAC128 mice striatal mitochondria exhibited higher H_2O_2 production rate. In cortex, H_2O_2 production was significantly increased in YAC128 mitochondria at 12mo, along with decreased catalase protein levels. Moreover, pre-symptomatic YAC128 striatal mitochondria exhibited an increase in both O_2 consumption rate and respiratory chain complexes I-IV activities, whereas a significant decrease in these parameters was observed in cortical YAC128 mitochondria. Mitochondrial Ca^{2+} handling also increased in YAC128 striatal mitochondria, but was reduced in the cortex of 3mo YAC128. In striatum of 9mo YAC128 mice an increase in mitochondrial coupling was observed, without changes in complexes I-IV activities. At old symptomatic stage (12mo) no major differences in mitochondrial function were observed either in cortex or striatal mitochondria. In sum, we found evidence for bioenergetics defects occurring in pre-symptomatic YAC128 mice, namely decreased mitochondrial function in the cortex and enhanced organelle activity in striatum, which is accompanied by *in vivo* decreased redox status.

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Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

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Johns Hopkins Solomon H. Snyder Department of Neuroscience Graduate Program

University of Rochester Discover Grant

NIH

Robert Packard Center at Johns Hopkins

Title: Mutant huntingtin disrupts the nuclear pore complex

Authors: *J. C. GLATZER[1], J. C. GRIMA[2], K. ZHANG[2], J. DAIGLE[2], Q. PENG[2], C. GEATER[3], I. AHMED[2], C. ROSS[2], W. DUAN[2], L. THOMPSON[3], S. H. SNYDER[2], J. D. ROTHSTEIN[2];
[1]Neurosci., Univ. of Rochester, Staten Island, NY; [2]Johns Hopkins Neurosci., Baltimore, MD; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Huntington's disease (HD) is caused by an expanded CAG repeat in the first exon of the huntingtin (htt) gene, resulting in progressive degeneration of striatal medium spiny neurons. Disease onset and severity are dependent on CAG repeat length with a longer expansion resulting in earlier onset and greater severity. The underlying mechanisms by which mutant htt causes the disease have not been fully elucidated. Recent studies suggest that nucleocytoplasmic transport dysfunction could be a pathogenic contributor.
The trafficking of RNA and proteins between the cytoplasm and the nucleus is a critical aspect of signal transduction and is especially arduous for neurons due to their highly polarized structure. This process is tightly regulated by Nuclear Pore Complexes (NPCs), which serve as the main gateway between the nucleus and cytoplasm. The NPC is one of the largest protein complexes in eukaryotic cells and consists of multiple copies of approximately 30 different protein subunits called Nucleoporins (NUPs). NUPs are organized into regions e.g. cytoplasmic ring/filaments, central channel, transmembrane, scaffold, nuclear ring/basket and each play roles in overall NPC transport function, such as mRNA export and protein import/export. Interestingly, it was recently discovered that some of the longest-lived proteins in the mammalian brain are NUPs located in the scaffold of the NPC and may represent the "weakest link" in the aging proteome.
Recent live-cell imaging studies show that mutant htt demonstrates reduced dynamics and rates of nucleocytoplasmic transport. Cell culture and transgenic animal models display distortions in nuclear envelope and increases in the clustering of NPCs. Studies have demonstrated that mutant htt can preferentially bind certain components of the NPC and that cytoplasmic protein aggregates can interfere with nucleocytoplasmic transport of protein and RNA. And finally, recent studies show that RAN translation products, which have been shown to disrupt nucleocytoplasmic transport in C9orf72 ALS-FTD, are also produced in HD. We hypothesize that products of the mutant htt repeat expansion are likely to disrupt nucleocytoplasmic transport at the NPC. To this end, we assessed NPC integrity in various transgenic HD mouse tissues, HD iPS neurons and postmortem human HD brain regions. Our data indicate that select NUPs involved in nucleocytoplasmic trafficking are affected as evidenced by nuclear aggregation that co-localizes with mutant htt. Functional studie sin iPS cell are ongoing. This study suggests that the NPC is disrupted in HD and potentially other repeat-expansion diseases.

Disclosures: **J.C. Glatzer:** None. **J.C. Grima:** None. **K. Zhang:** None. **J. Daigle:** None. **Q. Peng:** None. **C. Geater:** None. **I. Ahmed:** None. **C. Ross:** None. **W. Duan:** None. **L. Thompson:** None. **S.H. Snyder:** None. **J.D. Rothstein:** None.

Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 417.05/W8

Topic: C.04. Movement Disorders

Support: DFG, CEMMA, GRK1789

seed fund grant no 584/14, EHDN

Title: Integrated mitochondrial function in human muscle biopsies of Huntington's Disease patients and in different tissues of the HdhQ111 mouse model

Authors: *K. S. LINDENBERG[1], E. BARTH[1], T. MERZ[1], A. GUMPP[1], A. WITTING[1], P. WEYDT[1], M. ZUEGEL[2], J. STEINACKER[2], G. LANDWEHRMEYER[1], E. CALZIA[3]; [2]Div. of Sports and Rehabil. Med., [3]Inst. of Anesthesiological Pathophysiology and Process Develop., [1]Ulm Univ., Ulm, Germany

Abstract: The neurodegenerative genetic disorder of Huntington's disease (HD) is characterized by mitochondrial impairments of the respiratory chain. The ubiquitous expression of the disease causing mutant huntingtin gene raises the question to which extent changes in mitochondrial respiration are evident in the human skeletal muscle. In addition characterization of mitochondrial respiration in the muscle might allow conclusions about respiratory status in the brain.

The integrated respiratory chain function of the human quadriceps vastus lateralis was measured by high-resolution respirometry in fine needle biopsies of four pre-symptomatic HD mutation carriers and seven controls. The respiratory parameters indicated a trend towards a reduction in the respiratory control ratio (RCR) of the HD carriers. In parallel, murine cortex, liver, soleus muscle and heart of male HD knock-in mice (Hdh^{Q111}), which show a very mild phenotype, were examined by the same method. Significant changes of the respiration were restricted to the liver and the cortex. In addition mitochondrial DNA copy number and citrate synthase activity were determined to quantify the mitochondrial mass, showing no differences. From the murine tissues mRNA levels of key enzymes characterized the mitochondrial metabolic pathways. We demonstrated the feasibility to perform high-resolution respirometry measurements from fine needle human HD muscle biopsies. Furthermore, we conclude that differences in respiratory parameters of pre-symptomatic human muscle biopsies are rather limited, which is confirmed by the analysis of murine skeletal muscle tissue. The murine cortex and liver turned out to show respiratory changes in the Hdh^{Q111} mouse model, which indicates that respiratory capacities are different between tissues.

Disclosures: **K.S. Lindenberg:** None. **E. Barth:** None. **T. Merz:** None. **A. Gumpp:** None. **A. Witting:** None. **P. Weydt:** None. **M. Zuegel:** None. **J. Steinacker:** None. **G. Landwehrmeyer:** None. **E. Calzia:** None.

Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

Support: NIH NINDS R01 NS079450

Title: Cortical mitochondria from R6/2 Huntington's disease mice have elevated iron and altered proteomic and functional markers.

Authors: ***S. AGRAWAL**, J. H. FOX;
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Abstract: Huntington's disease (HD) is a progressive autosomal dominant disorder caused by an expanded CAG repeat in exon 1 of the huntingtin gene. Dysregulation of iron homeostasis and energetic dysfunction are consistent features in HD mouse models. However, it is unclear if there is a connection between iron accumulation and energetic dysfunction in HD. The present study aimed to assess the relationship between mitochondria and iron dysregulation in R6/2 HD mice. Studies were completed in 12-week-old HD mice that had advanced HD and wild-type (WT) litter-mates. Inductively-coupled-plasma mass spectrometry (ICP-MS) analysis of iron levels in purified mitochondria revealed a 51.6% increase in HD mice ($p=0.0020$) compared to controls. We used a number of outcomes to characterize mitochondrial state. Mitochondrial membrane potential and ATP level were decreased by HD ($p=0.0086$ and 0.0120, respectively). Two-dimensional electrophoresis with matrix-assisted-laser-desorption-ionization (MALDI)-time-of-flight (TOF)/TOF was used to identify differentially expressed mitochondrial proteins. Preliminary analysis has identified seventeen proteins including peroxiredoxin 3, DJ-1, ATP synthase sub-unit β and isocitrate dehydrogenase 3 alpha. These proteins have functions linked with energy metabolism, carbohydrate metabolism, redox cycling, protein folding, the ubiquitin proteasome system and apoptosis. We conclude that elevations of iron in HD brain mitochondria are consistent with preferential accumulation in this organelle. Findings demonstrate co-localization of iron dysregulation and measures of mitochondrial dysfunction in R6/2 HD brain.

Disclosures: **S. Agrawal:** None. **J.H. Fox:** None.

Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

Support: NIH

HDF

NeuroCure (German Research Foundation)

Title: Epigenetic and transcriptional dysregulation in prodromal Huntington's disease

Authors: *F. YILDIRIM[1], C. W. NG[2], J. CABOCHE[4], D. E. HOUSMAN[2], E. FRAENKEL[3]; [1]Dept. of Psychiatry and Psychotherapy & NeuroCure, Charité Med. Sch., Berlin, Germany; [2]Dept. of Biol., [3]Dept. of Biol. Engin., MIT, Cambridge, MA; [4]Unité Neurosciences, Paris Seine INSERM, Paris, France

Abstract: Transcriptional dysregulation is an early and pivotal feature of Huntington's disease that is strongly reflected in mouse models of this disorder. We have previously demonstrated in *in vitro* and mouse models genome-wide changes in DNA methylation and histone H3K4 trimethylation patterns that may underlie transcriptional dysregulation in HD (Vashishtha et al., 2013; Ng et al., 2013). In the study reported here, centering on the prodromal disease stage, we discovered that the pathogenic transcriptional and epigenetic alterations in the brain in mouse models in fact precede the overt onset of disease symptoms. Genome-wide analysis of transcription by RNA sequencing (RNA-Seq) revealed many key neuronal genes such as Drd1a and Drd2, Penk and Adora2a whose disruption are significant in HD pathogenesis to be down-regulated in the R6/1 mouse striatum as early as 8 weeks of age. RNA-Seq in a second mouse model that is the full length *Huntingtin* model of CHL2 [Hdh(CAG)150] heterozygous knock-in mice validated the ongoing transcriptional dysfunction in the striatum during prodromal HD and confirmed a highly significant overlap of differentially-expressed genes between the R6/1 and CHL2 models even at this early disease stage (142 overlapping genes, $p < 3e\text{-}128$). Comparison with a human HD study (Kuhn et al., 2007) showed that gene expression changes in our mouse models were consistent in the caudate nucleus of postmortem brains from HD patients (162 genes in R6/1 ($p < 3e\text{-}54$) and 51 genes in CHL2 ($p < 1.3e\text{-}10$)). This provides strong evidence that these mice models faithfully recapitulate the transcriptional dysfunction in human HD and that a significant portion of the early transcriptional changes persist throughout the later disease stages. Extension of our studies to genome-wide analysis of histone H3K27 acetylation, an active transcription and enhancer mark, by chromatin immunoprecipitation followed by sequencing (ChIP-Seq) revealed highly coordinated ($p< 1.7e\text{-}10$) histone acetylation changes along with the

detected transcriptional changes in the striatum of R6/1 mice at 8 weeks of age. By studying the DNA sequences that lie within the altered H3K27 acetylation regions, we identified specific transcriptional regulators whose activity may be most proximally-altered by mutant *Huntingtin* consequently leading to the earliest transcriptional changes that we detected by RNA-Seq. The potential therapeutic value of targeting these predicted transcriptional regulators is currently ongoing in mouse models of HD.

Disclosures: F. Yildirim: None. **C.W. Ng:** None. **J. Caboche:** None. **D.E. Housman:** None. **E. Fraenkel:** None.

Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

Support: NIH R01 NS076631

Title: Aberrant CELF1 expression in Huntington's disease brain contributes to alternative splicing and changes in RNA stability.

Authors: *S. RAMACHANDRAN[1], S. COFFIN[2], B. L. DAVIDSON[2];
[1]Pathology, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: CELF1 (CUGBP, Elav-Like Family Member 1) binds to highly conserved GU-rich elements (GREs) on target mRNAs and regulates gene expression by altering alternative splicing (AS) and RNA stability. CELF1's targets include RNA-binding proteins, transcription factors, and genes involved in neuronal differentiation, apoptosis, metabolism, and signal transduction. Evidence from invertebrate and vertebrate species implicate CELF1 in neurodegeneration, but it's role in normal or disease brain is not clear. Deep RNA-seq analysis of human Huntington's disease (HD) and control brains revealed significant up-regulation of CELF1 expression in HD samples. Further investigation showed that CELF1 protein is hyper-phosphorylated with increased stability and levels compared to controls in (i) the BA4 motor cortex from 20 HD patients, (ii) mature human neurons differentiated from iPSCs derived from 5 HD patients, (iii) striatum and cortex of the N171-82Q mouse model of HD (n=6), and (iv) cultured striatal neurons (STHdh-111Q). Furthermore, we found that CELF1 directly targets MBNL1 (Muscleblind-Like Splicing Regulator 1) mRNA for degradation, resulting in significantly reduced MBNL1 splicing activity in HD samples. We hypothesize that increased CELF1 activity

contributes to HD pathogenesis by altering expression of its splicing and RNA-stability targets. To address this we focused on the regulatory functions of CELF1 in the cortex of human HD brains and in the brains of HD mice models. Motif enrichment analysis of our RNA-seq data and candidate validation by high-throughput qPCR in CELF1 and MBNL1 knockout neuronal lines allowed prioritization of 120 direct targets of CELF1-mediated RNA stability or CELF1/MBNL1-mediated alternative splicing. First, RNA levels of CELF1-RNA stability targets were measured in brains from HD patients and mouse models, and those that were significantly different were verified by measuring mRNA decay in human and mouse neurons by 4sU-tagging. Next, we quantified the relative abundance of exons that are alternatively spliced by CELF1, MBNL1, or both in brains from HD patients and mouse models. Interestingly, reducing mutant Huntingtin levels in HD mice normalized expression of CELF1, MBNL1, and that of their respective target genes. Finally, to assess the physiological impact of increased CELF1 expression on HD pathogenesis, on going experiments aim to study the possible behavioural and histological benefits of reducing CELF1 expression in the striatum and cortex of the N171-82Q mouse model of HD.

Disclosures: **S. Ramachandran:** None. **S. Coffin:** None. **B.L. Davidson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder Spark Therapeutics.

Poster

417. Huntington's Disease Mechanisms II

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Support: NIH grant 5 RO1 NS038194-15

NIH grant 1 F30 NS095450-01

Title: Disease-associated changes in huntingtin mRNA 3'UTR isoform abundance

Authors: ***L. ROMO**[1], E. PFISTER[1], A. ASHAR-PATEL[2], M. MOORE[2], N. ARONIN[1]; [1]Med., [2]Umass Med. Sch., Worcester, MA

Abstract: Huntington’s disease is a devastating neurodegenerative disorder caused by an expanded glutamine repeat within exon 1 of the huntingtin (HTT) gene. The wild-type and mutant allele are expressed as two alternatively polyadenylated mRNA isoforms with tissue-specific abundance. The isoforms differ only in the length of their 3′ untranslated region

(3′UTR), and both are processed into the HTT protein. Recent findings indicate mutant and wild-type mRNA exhibit different localization and stability. These differences may be due to alternative processing of mutant and wild-type mRNA into different isoforms. Here, we aimed to determine if the abundance of HTT 3′UTR isoforms changes in patient cells and brain tissue. Quantitative PCR assessed the abundance of the HTT long 3′UTR isoform mRNA in control and patient cortex and cerebellum, fibroblasts, and neural stem cells. We found patient cerebellum and motor cortex contain a higher abundance of long HTT isoform than controls. However, patient fibroblasts and neural stem cells contain a significantly lower abundance of long HTT isoform than controls. Allele-specific qPCR showed isoform changes originate from both the mutant and wild-type alleles. To determine if the isoform change was specific to HTT, we performed next generation polyadenylation (polyA) site sequencing on patient and control cerebellum and motor cortex. We found a previously unknown mid-3'UTR isoform of HTT, which is also conserved in mice. Of 4813 genes with two detected 3'UTR polyA sites in the cerebellum, only 6 exhibit significantly ($p<0.01$) different isoform ratios between patients and controls. In the motor cortex, 49 out of 5776 exhibit significantly different isoform ratios. Isoform shifts are likely not due to global or HTT-specific changes in gene expression. Disease-associated expression changes are opposite to HTT isoform changes: greater in the motor cortex (574/14194) than the cerebellum (12/13361). Additionally, HTT is not significantly differently expressed between patients and controls. These data indicate disease-associated changes in HTT isoform abundance are unique to HTT and unrelated to transcriptional changes.
Our studies reveal a new isoform of HTT mRNA and differences in HTT mRNA isoform abundance in patient brain and cell lines. Differences in HTT mRNA 3′UTR isoform abundance could explain different stability and localization of the mutant and wild-type allele. Also, the long 3′UTR may be the ideal target for small RNAs aiming to degrade mutant but not wild-type HTT mRNA in the brain.

Disclosures: **L. Romo:** None. **E. Pfister:** None. **A. Ashar-Patel:** None. **M. Moore:** None. **N. Aronin:** None.

Poster

417. Huntington's Disease Mechanisms II

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Title: Recruitment of a transcriptional repressor to protein aggregates leads to de-repression of a pro-apoptotic gene activity and contributes to neuronal toxicity in polyglutamine diseases.

Authors: Z. S. CHEN[1], S. PENG[1], Q. ZHANG[1], D. D. RUDNICKI[2], *E. CHAN[1];
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Abstract: Polyglutamine (polyQ) diseases are a growing group of dominant heritable neurodegenerative diseases caused by the expansion of glutamine-encoding CAG repeats located within the coding region of the affected genes. Expression of the mutant disease genes results in the biosynthesis of elongated polyQ disease proteins. Protein aggregate formation is a pathogenic hallmark of polyQ diseases. Polyglutamine aggregates recruit essential proteins in affected neurons, which leads to the cellular depletion of these aggregate-recruited proteins (ARPs) and results in perturbation of neuronal cell function. Transcriptional dysregulation caused by APRs has been reported in several polyQ diseases. Here, we demonstrate the sequestration of a transcriptional repressor protein to polyQ aggregates in the brain tissues of polyQ patients. We showed that the depletion of this repressor protein causes the transcriptional de-repression of a pro-apoptotic gene expression in cellular disease models and subsequently leads to neuronal apoptotic cell death. In summary, our investigation unveils a polyQ pathogenic pathway that involves a transcriptional repressor-triggered promotion of apoptotic cell death in polyQ disease. Targeting this novel pathway may be therapeutically beneficial.

Disclosures: Z.S. Chen: None. **S. Peng:** None. **Q. Zhang:** None. **D.D. Rudnicki:** None. **E. Chan:** None.

Poster

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NIH P50MH096890

NIA 1T32AG049688-01

Title: The implications of H3K9me3 in neuronal dysfunction underlying Huntington’s disease

Authors: *S. CHANDRASEKARAN[1], Y. JIANG[1], E. LOH[2], R. H. MYERS[3], L. SHEN[2], S. AKBARIAN[1];
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Abstract: The human epigenome includes numerous histone post-translational modifications (PTMs) that confer varied chromatin states to modulate gene expression. As many PTMs are as-of-yet uncharacterized with respect to their roles in the brain, we began by exploring the involvement of one such mark -- the globally repressive histone 3 tri-methyl lysine 9 (H3K9me3) -- in cell-type-specific neuroepigenomic regulation in mouse cerebral cortex. Additionally, considering preliminary postmortem human data suggesting a pervasive 'derepression' differentiating Huntington's patients from controls, we sought to corroborate any H3K9me3-related findings from our mouse dataset in a postmortem brain cohort of Huntington's cases and controls. Nuclei from four wild type mouse cortices and four postmortem human tissue samples (Brodmann Area 9; two diseased, two age-matched controls) were sorted into neuronal and non-neuronal ("glial") populations using fluorescence-activated nuclei sorting. Following MNase digestion, chromatin immunoprecipitation with anti-H3K9me3 antibody was performed to isolate bound DNA fragments. Samples were ultimately submitted for high-throughput sequencing. Using BowTie2, retrieved sequencing files were aligned to the respective reference genomes (mm10 mouse, hg19 human); diffReps was used to annotate differential regions between the neuronal and glial subsets. Neuronal and glial H3K9me3 in the mouse ChIP-seq tracks showed significant differences in genome-wide distribution; further breakdown into genomic element categories revealed distinct patterns distinguishing the two populations, a majority of which occurred in "gene-poor" gene deserts and intergenic regions. Appreciable differences at these "gene-poor" regions in the neuronal and glial populations were also discernable between Huntington's and control samples. In sum, the above results about cell-type specific differences in H3K9me3 epigenomic profiles offer promising leads about the underlying importance of this PTM -- diseases with hallmarks of neuronal dysfunction could be closely linked with aberrant cell-type specific H3K9me3 landscapes. Ongoing work with rare asymptomatic Huntington's brains could further elucidate key neuroepigenetic determinants in this disease.

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Poster

417. Huntington's Disease Mechanisms II

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Title: Pias1 regulates mutant huntingtin accumulation and huntington's disease-associated phenotypes *In vivo*

Authors: *J. OCHABA[1], A. MAS MONTEYS[3], E. L. MOROZKO[1], J. G. O'ROURKE[4], J. C. REIDLING[2], J. S. STEFFAN[2], B. L. DAVIDSON[3,5], L. M. THOMPSON[2];
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Abstract: The disruption of protein quality control networks is central to pathology in Huntington's disease (HD) and other neurodegenerative disorders. The aberrant accumulation of insoluble high molecular weight protein complexes containing the huntingtin (HTT) protein and SUMOylated protein corresponds to disease manifestation. We previously identified a HTT selective E3 SUMO ligase, PIAS1, which regulates HTT accumulation and SUMO modification in cells. Here we investigated whether PIAS1 modulation in neurons alters HD-associated phenotypes *in vivo*. Instrastriatal injection of a PIAS1-directed miRNA significantly improved behavioral phenotypes in rapidly progressing mutant HTT (mHTT) fragment R6/2 mice. PIAS1 reduction prevented the accumulation and aggregation of mHTT, SUMO- and ubiquitin-modified proteins, increased synaptophysin levels, and normalized key inflammatory markers. In contrast, PIAS1 overexpression exacerbated mHTT-associated phenotypes and aberrant protein accumulation. These results confirm the association between aberrant accumulation of expanded polyglutamine-dependent insoluble protein species to pathogenesis and link phenotypic benefit to reduction of these species through PIAS1 modulation. Further, it suggests that PIAS1 may link protein homeostasis and neuroinflammation in HD through a combination of modulating or compensating for dysfunctional inflammatory signaling cascades between neurons and microglia, and modulating accumulation of toxic HMW species of HTT, potentially allowing improved homeostasis and flux through protein clearance pathways.

Disclosures: **J. Ochaba:** None. **A. Mas Monteys:** None. **E.L. Morozko:** None. **J.G. O'Rourke:** None. **J.C. Reidling:** None. **J.S. Steffan:** None. **B.L. Davidson:** None. **L.M. Thompson:** None.

Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

Support: GACR P304/12/G069

Title: Structural tissue changes and their effect on brain diffusion in the R6/2 mouse model of Huntington's disease

Authors: *L. VARGOVA[1,2], I. VORISEK[2], M. SYKA[2];
[1]Dept. of Neurosci., Charles University, 2nd Fac. of Med., Praha, Czech Republic; [2]Dept. of Neurosci., Inst. of Exptl. Med., Prague, Czech Republic

Abstract: Huntington's disease (HD) is a fatal human hereditary neurodegenerative disorder, caused by a mutation of the IT 15 gene. Selective degeneration of neurons and severe atrophy of the striatum result in progressive immobility, dementia and premature death. Magnetic resonance (MR) is often used to assess the progression of the disease. However, the MR results are more or less inconsistent, probably due to metalloprotein-bound iron accumulation in the striatum (1). To explore the background for MR changes in HD, we employed two different techniques to examine changes in the diffusion properties of the globus pallidus (GP) and the somatosensory cortex in the R6/2 mouse model of HD (HU) and in wild type controls (WT). The apparent diffusion coefficient of water (ADC_W) was determined by MR *in vivo*, while the extracellular space volume fraction α (α = extracellular volume/total tissue volume) and the tortuosity λ (λ^2 = free/apparent diffusion coefficient) were measured using the real-time iontophoretic (RTI) method *in situ*. In a potentially anisotropic globus pallidum, diffusion measurements were performed in the mediolateral (x), rostrocaudal (y) and ventrodorsal (z) axes. The results were correlated with histological analysis of the tissue.Diffusion in the GP was anisotropic, with preferential diffusion in the y axis in both WT and HU mice. Values of α were significantly lower in WT than in HU animals, while no significant difference was found in the values of tortuosity. Values of ADC_W in all axis were significantly higher in HU mice than in WT animals. Staining for chondroitinsulphate proteoglycan (CSPG) revealed a decrease in the relative fluorescent signal intensity by 28% in HU mice compared to control animals. At the same time, the number of NeuN positive neurons decreased to 73% of controls. Staining for GFAP reflects

astroglitic-like changes in astrocytes in the GP of HU mice. However, the ratio of GFAP-positive cells to the total cell count did not increase significantly. No significant differences between HU and WT animals in any of the studied parameters and variables were found in the cortex.Our results suggest that in the R6/2 model of HD, structural and diffusion changes are primarily expressed in the GP, not in the cortex. In the HU mice, a profound decrease in the number of neurons resulted in increased ADC_W and α values, while the expected decrease in tortousity λ due to enlarged α and loss of CSPG was prevented by an increase of diffusion barriers formed by the structural changes of astrocytes.
(1) Syka et al., 2015, PlosOne

Disclosures: L. Vargova: None. **I. Vorisek:** None. **M. Syka:** None.

Poster

417. Huntington's Disease Mechanisms II

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Topic: C.04. Movement Disorders

Support: CHDI Foundation

Title: Age-related changes in cortical and striatal NADPH-diaphorase staining in the Q175 mouse model of Huntington's disease.

Authors: *F. E. PADOVAN NETO, L. JURKOWSKI, C. MURRAY, A. WEST;
Neurosci., Rosalind Franklin Univ., North Chicago, IL

Abstract: In Huntington's disease (HD), corticostriatal and striatopallidal projection neurons preferentially degenerate as a result of mutant huntingtin expression and accumulation of polyglutamine inclusion bodies. Additional evidence points to abnormalities in striatal nitric oxide (NO) synthesis, cyclic nucleotide production, and phosphodiesterase expression which may be linked to deficits in corticostriatal transmission. The underlying mechanisms remain unknown, however, our recent studies in neuronal NO synthase (nNOS) knockout mice demonstrated that striatal projection neurons were substantially less responsive to cortical drive as compared to wild type (WT) controls, indicating that tonic NO acts to facilitate corticostriatal transmission in the intact striatum. Thus, deficits in corticostriatal transmission observed in HD could be associated with decreased nNOS activity in cortical-striatal circuits. Numerous labs have now shown that quantification of NADPH-diaphorase (NADPH-d) staining in striatum using optical density measures is an accurate index of nNOS activity. Because nitrergic interneurons are largely spared in the HD brain, we utilized NADPH-d histochemistry to assess

nNOS activity in male and female Q175 heterozygous mice (16 and 21 months of age). The impact of genotype and age was assessed in these animals using two-way ANOVA and Bonferroni post-hoc tests. In motor cortex, an overall effect of genotype on NADPH-d staining was observed between WT and Q175 het mice at 21 but not 16 months of age. Post-hoc comparisons revealed a significant decrease in NADPH-d staining at 21 months of age in male Q175, but not female mice as compared to WT controls. Also, an overall effect of genotype on striatal NADPH-d staining was observed between WT and Q175 mice at both 16 and 21 months of age. Post-hoc comparisons in 16 month old groups revealed no differences in NADPH-d staining across gender. Post-hoc comparisons in 21 month old groups revealed a trend towards a decrease in NADPH-d staining in males compared to WT controls, but no significant changes were detected in females. In summary, these data point to an age-dependent deficit in nNOS activity in the cortex and striatum in the Q175 mouse model of HD. Furthermore, males may be more susceptible to changes in nNOS activity in the cortex and striatum during aging. Taken together with previous studies, these observations indicate that decreased nitrergic transmission might contribute to circuit pathophysiology in HD, and that these deficits could be associated with disruptions in corticostriatal transmission observed in this devastating disease.

Disclosures: F.E. Padovan Neto: None. **L. Jurkowski:** None. **C. Murray:** None. **A. West:** None.

Poster

417. Huntington's Disease Mechanisms II

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CHDI

Title: Mechanisms underlying axonal transport deficits in Huntington's disease.

Authors: *M. KANG[1,2], R. GATTO[3], C. WEISSMANN[3], N. MESNARD-HOAGLIN[3], S. T. BRADY[3,2], G. A. MORFINI[3,2];
[1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Marine Biol. Lab., Woods Hole, MA; [3]Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Huntington’s disease (HD) is a fatal adult-onset neurodegenerative disease that results from expansion of a polyglutamine tract located at the amino terminus of the protein huntingtin

(htt). Consistent with the early deficits in synaptic and neuritic connectivity observed in HD, several independent studies documented deficits of fast axonal transport (FAT) in cellular and animal models of HD. However, a molecular basis for these deficits has not been established. Using the isolated squid axoplasm preparation, our prior work showed that pathogenic mutant htt (*mhtt*) inhibits FAT by promoting abnormal activation of c-Jun amino-terminal kinase 3 (JNK3). Further, these studies revealed that JNK3 directly phosphorylates kinesin-1 subunits of the motor protein conventional kinesin, inhibiting its microtubule-binding activity, but the relevance of these findings to mammalian neurons has not been established.
To evaluate the contribution of JNK3 to mhtt-induced deficits in FAT in a mammalian system, we generated R6/2-JNK3$^{-/-}$ mice. Remarkably, genetic ablation of JNK3 dramatically ameliorated R6/2 mice neuropathology and survival. Based on these observations, we performed an analysis of various FAT components in R6/2 and R6/2-JNK3$^{-/-}$ neurons. Collectively, the available data strongly suggests that JNK3-dependent alterations in the phosphorylation of major motor proteins responsible for FAT represent an important component of HD pathology.

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Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 417.16/X7

Topic: C.04. Movement Disorders

Title: MID1-RNA interactions: implications in Huntington's disease.

Authors: *J. SCHILLING[1], A. DAGANE[2], I. ATANASSOV[3], E. WANKER[2], S. KRAUß[1]; [1]DZNE, Bonn, Germany; [2]Max Delbrueck Ctr., Berlin, Germany; [3]MPI for Biol. of Aging, Cologne, Germany

Abstract: Huntington’s disease (HD) is a hereditary neurodegenerative disorder leading to physical, mental and behavioral disabilities. The mutant gene product is characterized by an elongated stretch of CAG repeats in its first exon that transcribes into an extended sequence of polyglutamines within the Huntingtin (HTT) protein. This causes the protein to aggregate and to exert pathogenic effects on neurons. Additionally, mutant HTT RNA is known to be neurotoxic by itself. The mutation produces a stable hairpin in the transcript capable of enhancing translation in an *in vitro* reporter assay. Furthermore, this repeat-dependent translational induction is regulated by the MID1 protein complex. MID1 is a E3 ubiquitin ligase that affects translation initiation of multiple transcripts by interfering with mTOR and PP2A signaling. To

understand how the MID1 complex together with mutant HTT RNA is involved in the development of HD, we characterized their *in vitro* protein interactions by quantitative mass spectrometry. The two datasets show substantial overlap: 25% of the identified proteins are shared binding partners. These proteins are for example involved in RNA processing, splicing and translation. To shed light on their involvement in the pathophysiology we are characterizing common and unique candidates using *in vitro* and *in vivo* models of HD. Additionally, by mapping the MID1-RNA binding site we can study the binding mode of MID1 to HTT RNA. This could help to explain the mechanism underlying the translational induction of repeat RNA.

Disclosures: J. Schilling: None. **A. Dagane:** None. **I. Atanassov:** None. **E. Wanker:** None. **S. Krauß:** None.

Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 417.17/X8

Topic: C.04. Movement Disorders

Title: Yap and mst1 dysfunction: implications for transcriptional dysregulation in huntington's disease

Authors: *A. DIOS[1], K. MUELLER[1], K. GLAJCH[1], M. HUIZENGA[1], M. LAQUAGLIA[2], K. VAKILI[2], G. SADRI-VAKILI[1];
[1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Dept. of Surgery, Boston Children's Hosp., Boston, MA

Abstract: The Hippo/YAP signaling pathway has been implicated in mammalian organ size regulation and tumor suppression. Specifically, this pathway plays a critical role in regulating the activity of the transcriptional co-activator Yes-associated protein (YAP), which modulates a proliferative transcriptional program by binding to the transcription factor TEAD. While the Hippo/YAP pathway is known to be inhibited during tumorigenesis, recent studies have revealed that it may also play a role in neurodegeneration. Specifically, while mammalian sterile 20 (STE20)-like kinase 1 (MST1), an upstream pro-apoptotic protein kinase in the Hippo pathway, reduces YAP activity and mediates oxidative stress-induced neuronal death, increases in YAP activity provide neuroprotection. Therefore, we investigated the possible role of this pathway in Huntington's disease (HD) pathogenesis. Our results demonstrate that there is a significant increase in phosphorylated MST1 (pMST1), the active form, in post-mortem human cortex from HD patients. Additionally, pMST1 was also increased in the striatum and cortex of $Hdh^{111/111}$ mice compared to control. There was also a significant and concomitant increase in

phosphorylated YAP, the inactive form, in HD post-mortem cortex as well as in $Hdh^{111/111}$ striatum. While YAP was expressed in both neurons and glia in post-mortem brain, YAP nuclear localization was decreased in HD compared to control cortex. Lastly, TEAD/YAP interactions were decreased in HD and there was a decrease in immunoprecipitated DNA following TEAD chromatin immunoprecipitation in HD brain, suggesting decreased transcriptional activity. Together, these results demonstrate that while MST1 is activated in HD nuclear YAP is decreased. Thus, Decreases in nuclear YAP and TEAD interactions could significantly contribute to the pathogenic mechanism in HD by altering gene expression.

Disclosures: A. Dios: None. **K. Mueller:** None. **K. Glajch:** None. **M. Huizenga:** None. **M. LaQuaglia:** None. **K. Vakili:** None. **G. Sadri-Vakili:** None.

Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

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Program#/Poster#: 417.18/X9

Topic: C.04. Movement Disorders

Support: Huntington's Disease Society of America

Title: Huntingtin protein interactions evaluated in systems biology analyses among non-human animal models of Huntington's disease (HD) reveals the broad scope of neuronal functional protein networks potentially involved in HD.

Authors: *S. PODVIN[1], V. HOOK[2];
[1]Skaggs Sch. of Pharm. and Pharmaceut. Sci., UCSD, LA Jolla, CA; [2]Skaggs Sch. of Pharm. and Pharmaceut. Sciences, Dept. of Neuroscience, Dept. of Pharmacol., UCSD, La Jolla, CA

Abstract: Huntington's disease is a genetic neurodegenerative disease caused by trinucleotide repeat (CAG) expansions in the HTT gene encoding the huntingtin protein with increased polyglutamine (polyQ) tract, causing motor dysfunction and cognitive deficits. Adult HD occurs in the range of ~38 to 50-55 repeats within the HTT gene, compared to the normal range of ~10-36 repeats. Moreover, juvenile HD patients with >60 repeats display very early age of onset in children. Numerous HD animal models from yeast to transgenic mice have investigated proteins interacting with mutant huntingtin to gain insight into the initial network of Htt interactions that may participate in HD. It will be of benefit to the field to analyze the entirety of Htt protein interaction data in the field (represented by more than ~250 published articles) to gain understanding of the breadth of possible protein interaction mechanisms initiated by mutant Htt compared to normal Htt, related to the HD disease process. For these reasons, this study

compiled Htt protein interaction observed in non-human animal models of yeast, Drosophila, mice, and related. Analyses includes ranges of HTT repeat numbers and Htt forms - full-length or fragments - investigated for defining Htt protein interactions. Systems biology analyses using Cytoscape defines functional categories of proteins interacting with Htt forms and proposes possible Htt protein interaction networks based on protein interaction database analyses. Htt protein interaction networks analyses illustrates the complexity of multiple neuronal functions involved including intracellular trafficking and transport, synaptic cell-cell communication and interactions, energy metabolism, cell viability, and gene/protein expression. Systems biology analyses among multiple HD models can together provide insight into mutant Htt protein interactions potentially involved in HD.

Disclosures: **S. Podvin:** None. **V. Hook:** None.

Poster

417. Huntington's Disease Mechanisms II

Location: Halls B-H

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Topic: C.04. Movement Disorders

Support: NIH Grant R21NS078643

NIH Grant R21NS083365

NIH Grant U24NS078370

Title: Comparative study of HDL2 versus HD iPSC models

Authors: ***S. AKIMOV**[1], D. RUDNICKI[1], M. ENCARNACION[2], X. SUN[3], D. SAREEN[4], C. A. ROSS[1], R. L. MARGOLIS[1];
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Abstract: Huntington’s Disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by expanded CAG repeats in exon 1 of the *Huntingtin* (*HTT*) gene. Huntington disease-like 2 (HDL2), discovered and genetically defined by the Margolis group, is a rare, autosomal dominant neurodegenerative disorder, with remarkable clinical, genetic, and pathological similarities to HD. Neuropathology of both disorders is characterized by cortical and striatal degeneration and the presence of neuronal protein aggregates. HDL2 is caused by a CTG/CAG

expansion on chromosome 16q24. Normal alleles contain 6-28 triplets, while pathogenic repeats range from 40-59 triplets. In the CTG orientation, the repeat falls in the gene *junctophilin-3* (*JPH*3). Insight gained from HDL2 have facilitated the discovery of an antisense gene at the HD locus, the role of mutant HTT transcripts in HD neurotoxicity, and potential RNA processing deficits in HD. We have hypothesized that the HDL2 mutation leads to neurodegeneration via a combination of loss of *JPH3* expression, RNA toxicity of the sense strand transcript containing an expanded CUG repeat, and expression of polyglutamine from a cryptic gene on the antisense strand. To test this hypothesis we developed HDL2 iPSCs using the same non-integrating episomal method that we have used to generate HD iPSCs (HD iPSC Consortium, Cell Stem Cell, 2012). Here, we demonstrate that HDL2 iPSCs differentiate to neural phenotypes expressing markers of mature neurons. Neural progenitors derived from HDL2 iPSCs express wild-type and mutant *JPH3* message. HDL2 iPSCs differentiated to striatal neurons express less *JPH3* transcript than similarly differentiated control or HD iPSCs, consistent with studies of post-mortem HDL2 brain. Aggregation of *JPH3* transcripts into RNA foci was observed in differentiated HDL2 iPSCs, similar to the foci observed in HDL2 post-mortem brain. BDNF withdrawal increased death of differentiated HDL2 iPSCs compared to differentiated control iPSCs, quantitatively similar to the toxicity observed after BDNF withdrawal in differentiated HD iPSCs. As in HD iPSC cells, BDNF withdrawal-induced toxicity of differentiated HDL2 iPSCs was rescued by BDNF and by 7,8-dihydroxyflavone (*7,8-DHF*), a TrkB small molecule agonist. These results provide initial validation for our iPSC model of HDL2, and suggest the likelihood that HD and HDL2 share one or more pathogenic pathways. Identifying these common pathways may provide important leads in on-going efforts to find therapeutic targets in HD, HDL2 and, possibly, other neurodegenerative disorders.

Disclosures: **S. Akimov:** None. **D. Rudnicki:** None. **M. Encarnacion:** None. **X. Sun:** None. **D. Sareen:** None. **C.A. Ross:** None. **R.L. Margolis:** None.

Poster

417. Huntington's Disease Mechanisms II

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Program#/Poster#: 417.20/X11

Topic: C.04. Movement Disorders

Support: NSFC81271259

Title: Uncovering mechanisms of gaba neuron degeneration using huntington's disease ipscs

Authors: ***L. MA**[1], H. SAIYIN, 200433[2], X. WANG[2], L. GAO[3], J. LI[3], S.-C. ZHANG[4], L. MA[3];

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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder cuased by an abnormal expansion of CAG repeats. It is presently unknown how the expanded CAG repeats cause degeneration, preferentially in striatal medium spiny GABA neurons. It has no effective treatment. The present proposal, building upon our successful establishment of iPSC lines from a cohort of HD families and our ability to differentiate human iPSCs to enriched populations of functional medium spiny GABA projection neurons, will attempt to uncover the cellular and molecular mechanisms underlying GABA neuron degeneration in HD. We revealed the pathological phenotypes of HD in GABA and other neuronal types that are derived from HD iPSCs in vitro. Our study will most likely build a human disease model for further mechanistic exploration and for drug discovery. It may also reveal potential mechanisms of preferential GABA neuronal degeneration caused by CAG reapeats.

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Poster

417. Huntington's Disease Mechanisms II

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Program#/Poster#: 417.21/X12

Topic: C.04. Movement Disorders

Support: NRF(2014R1A1A4A01003859)

KHT R& D(HI14C1891)

Title: Inhibition of nonmuscle myosinIIB facilitates polyQ-aggregates and cellular toxicity in primary neurons and HD- iPSC-derived neurons

Authors: Y.-K. LEE[1], W.-K. JUNG[1], H.-E. CHOI[1], M.-H. JUN[1], Y. HUH[2], D.-J. JANG[3], *J.-A. LEE[4];
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Abstract: Huntington's disease (HD) whose symptoms are uncontrolled movements, emotional problems, and impairment of cognition caused by expansion and protein aggregates of a polyglutamine (poly-Q) repeat in the huntingtin protein. In the course of the disease, expanded

huntingtin accumulates as the heterogeneous forms of aggregates such as oligomers, fibrils, and large inclusion. However, how aggregation of polyQ-aggregates is regulated is largely unknown. In this study, we found that myosinIIB, a non-muscle-type of myosin as a molecular actin-based motor protein, was strikingly redistributed into large polyQ-aggregates in cells overexpressing EGFP-tagged N-terminal fragment of htt with 72 or 150 glutamine residues (Nhtt150Q-EGFP). Furthermore, we found that FUS and TDP-43 which are associated with frontotemporal dementia and amyotrophic lateral sclerosis was sequestered into polyQ-myosinIIB aggregates indicating their roles on cellular aggregation. To investigate the role of myosinIIB in poly-Q aggregation and cellular toxicity with blebbistatin, an inhibitor of myosinIIB, or myosinIIB siRNA was incubated in cells expressing Nhtt150Q-EGFP or Nhtt16Q-EGFP. Very interestingly, inhibition of myosinIIB by blebbistatin or siRNA facilitated accumulation of small size of soluble polyQ aggregates compared to control cells. Furthermore, inhibition of myosinIIB in Nhtt15OQ-expressing neurons enhanced cellular toxicity induced by the ER-stress in primary cortical neurons and HD-iPSC-derived neurons. Taken together, these data suggest that myosinIIB is involved in the process of formation of small poly-Q aggregates which is toxic to neurons under ER stress. Therefore, our study provides a novel role of myosinIIB as a regulator on poly-Q aggregation and ER-stress induced neuronal toxicity.

Disclosures: **Y. Lee:** None. **W. Jung:** None. **H. Choi:** None. **M. Jun:** None. **Y. Huh:** None. **D. Jang:** None. **J. Lee:** None.

Poster

418. Huntington's Disease Therapeutics

Location: Halls B-H

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IBRO International Travel Grants 2016 Funding Program

Title: Exacerbated SIRT3 activity alters mitochondrial function and dynamics in Huntington's disease models

Authors: *L. NAIA[1,2], C. CARMO[1], A. M. OLIVEIRA[1], J. VALERO[1,3], C. LOPES[1], C. R. OLIVEIRA[1,2], T. R. ROSENSTOCK[1,3], A. C. REGO[1,2];
[1]Center for Neuroscience and Cell Biology - CNC, Coimbra, Portugal; [2]Fac. of Medicine, Univ. of Coimbra, Coimbra, Portugal; [3]Inst. for Interdisciplinary Research, Univ. of Coimbra (IIIUC), Coimbra, Portugal

Abstract: Huntington's disease (HD) is a genetic neurodegenerative disorder linked to expanded polyglutamine tract in the huntingtin (HTT) protein. Striatal medium spiny neurons are the most affected population. Although the mechanisms by which neurons die are uncertain, mitochondrial dysfunction has been implicated in HD pathogenesis. Rescue of mitochondrial morphology by modulation of mitochondrial dynamics could help to restore mitochondrial function. Therefore, we speculated that targeting SIRT3, a stress responsive NAD^+-dependent mitochondrial lysine deacetylase, might influence these processes. In the present work we show that ST*Hdh*$^{Q111/Q111}$ striatal cells, expressing mutant Htt with 111 glutamines, and HD patients-derived human lymphoblasts and induced pluripotent stem cells exhibited an increase in SIRT3 protein and mRNA levels, along with increased SIRT3 enzymatic activity, in comparison with control cells. Symptomatic YAC128 HD transgenic mice also presented increased SIRT3 mRNA levels and deacetylation of superoxide dismutase 2, a pre-recognized mitochondrial SIRT3 target. Concordantly, co-localization analysis revealed that HD striatal cells display an increased accumulation of overexpressed SIRT3 (SIRT3OE) in mitochondria, when compared to wild-type cells, as well as decreased lysine acetylation in both genotypes. Failure to maintain mitochondrial membrane potential (mmp) is one feature of HD striatal cells that has been linked with impaired balance in mitochondrial fission and fusion. Concordantly, untransfected HD cells displayed mitochondrial loss along with increased number of cells exhibiting a fragmented mitochondrial network. Remarkably, the unbalance between mitochondrial fission and fusion observed in HD cells was reduced after SIRT3OE, with higher prevalence of tubular mitochondria due to decreased accumulation of Fis1 and Drp1 in mitochondria. Preliminary data from single-cell functional imaging further revealed that SIRT3OE improves mmp in both wild-type and mutant striatal cells. Nevertheless, protein assessment of autophagy modulators revealed no significant changes following SIRT3OE. Overall, we report that increased SIRT3 activity ameliorates mitochondrial dynamics in HD striatal cells culminating in enhanced mitochondrial function and prevention of neuronal damage in HD.

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Poster

418. Huntington's Disease Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 418.02/X14

Topic: C.04. Movement Disorders

Title: Progress towards potent, selective and brain penetrant Rho kinase inhibitors suitable for a proof-of-concept study in HD models

Authors: G. MCALLISTER[1], O. AZIZ[2], C. LUCKHURST[2], I. ANGULO-HERRERA[2], W. BLACKABY[2], J. FRANCIS[2], A. HAUGHAN[2], H. KEARNEY[2], J. LIEBESCHUETZ[2], S. L. MARTIN[2], K. MATTHEWS[2], V. BEAUMONT[3], R. CACHOPE[3], M. MAILLARD[3], M. ROSE[3], I. MUNOZ-SANJUAN[3], *C. DOMINGUEZ[4];
[1]Charles River, Saffron Walden, United Kingdom; [2]Charles River, Saffron Walden, United Kingdom; [3]CHDI Mgmt. Inc., Los Angeles, CA; [4]CHDI Managment Inc, Los Angeles, CA

Abstract: Rho kinases (ROCK) belong to the serine-threonine family and are involved in a variety of functions, axonal growth, synapse stability and migration. ROCK1 and 2 isoforms demonstrate high sequence similarity but differential tissue expression, with ROCK2 more prevalent in the CNS, and ROCK1 more abundant in peripheral organs. Abnormal activation of ROCK and downstream effectors has been reported in several CNS disorders, and it has been postulated that abnormal ROCK activation might play a role in the pathology of Huntington's disease. A number of ROCK inhibitors (ROCKi) exist including drugs such as fasudil and ripasudil for peripheral indications. Pharmacological ROCKi have been reported to have a benefit in neuronal degeneration, although no known ROCKi combine kinase selectivity with properties consistent with CNS exposure. We have developed a screening cascade designed to optimize compounds for ROCK biochemical and cellular potency, as well as monitor selectivity over the related kinases. Results from our lead compound, CHDI-00488367, demonstrate sustained brain exposure over the cellular IC50 following a single dose at 10 mg/kg. Samples from this study were profiled using KiNativ™ technology and showed that brain exposure correlated with target occupancy, giving us confidence that brain exposure would translate into an inhibition of ROCK activity at the intended site of action. These ROCKi have potential as tool compounds to assess whether they will reduce mHtt aggregation in an HD model system or have a positive impact on survival. Current efforts are targeted towards measurement of compound efficacy following *in vivo* dosing through a ROCK dependent mechanism. Efforts to develop an *ex vivo* biomarker to aid this approach will also be discussed.

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Cachope: None. **M. Maillard:** None. **M. Rose:** None. **I. Munoz-Sanjuan:** None. **C. Dominguez:** None.

Poster

418. Huntington's Disease Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 418.03/X15

Topic: C.04. Movement Disorders

Title: Potent, selective and brain penetrant ATM inhibitor suitable for a proof-of-concept study in HD models

Authors: O. LAZARI[1], P. BRECCIA[1], J. BATE[1], K. MATTHEWS[1], G. WISHART[1], H. VATER[1], S. L. MARTIN[1], H. COX[1], W. BLACKABY[1], G. MCALLISTER[1], D. YATES[1], *D. F. FISCHER[3], P. MILIANI DE MARVAL[2], L. TOLEDO-SHERMAN[4], R. CACHOPE[4], M. ROSE[4], I. MUNOZ-SANJUAN[4], C. DOMINGUEZ[4];
[1]Charles River, Saffron Walden, United Kingdom; [2]Charles River, Morrisville, NC; [3]Biofocus, Charles River, Leiden, Netherlands; [4]CHDI Foundation/Management Inc., Los Angeles, CA

Abstract: Emerging evidence shows that the Ataxia Telangiectasia Mutated kinase (ATM) signalling pathway is dysregulated in neurodegenerative disorders including Huntington's Disease (HD). ATM signalling has been shown to be elevated in cells derived from HD patients and mouse models of HD. Genetic and pharmacological evidence from cellular and animal models of HD suggests that reduction of ATM signalling can ameliorate mHTT toxicity. Thus an inhibitor of ATM kinase presents a promising therapeutic intervention strategy for the treatment of HD.We have developed a screening cascade designed to optimize compounds for ATM biochemical and cellular potency, as well as monitor selectivity over the related kinases ATR, DNA-PK and Vps34. Several promising series were identified, with appropriate properties for further studies. Results from our lead compound, CHDI-00485194, demonstrate sustained brain exposure over the cellular IC50 following a single dose at 100 mg/kg. Samples from this study were profiled using KiNativ™ technology and showed that brain exposure correlated with target occupancy, giving us confidence that brain exposure would translate into an inhibition of ATM activity at the intended site of action. These target engagement results were confirmed in a PK/PD biomarker study using WT mice dosed with the compound and challenged with 5 Gy of X-ray irradiation; here, a dose-dependent inhibition of radiation-induced phosphorylation of the ATM substrate, KAP1, was observed. Next steps are to move towards a full efficacy study with our lead compound in a HD mouse model.

Disclosures: **O. Lazari:** None. **P. Breccia:** None. **J. Bate:** None. **K. Matthews:** None. **G. Wishart:** None. **H. Vater:** None. **S.L. Martin:** None. **H. Cox:** None. **W. Blackaby:** None. **G. McAllister:** None. **D. Yates:** None. **D.F. Fischer:** None. **P. Miliani de Marval:** None. **L. Toledo-Sherman:** None. **R. Cachope:** None. **M. Rose:** None. **I. Munoz-Sanjuan:** None. **C. Dominguez:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: CIHR Doctoral award - CGS-D

Title: Therapeutic and disease-modifying effects of ganglioside GM1 in mouse models of Huntington's disease

Authors: ***M. ALPAUGH**, D. GALLEGUILLOS, L. C. MORALES, S. LACKEY, P. KAR, B. KERR, K. TODD, S. SIPIONE;
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Abstract: ***Background.*** Huntington disease (HD) is a neurodegenerative disorder caused by an expansion of a polyglutamine stretch in the huntingtin protein. The resulting mutant protein acquires toxic conformations and aggregates within the cells, leading to neuronal dysfunction and death. We previously showed that levels of ganglioside GM1, a lipid highly enriched in the brain, are decreased in HD models. Administration of exogenous GM1 resulted in normalization of motor behavior in a transgenic model of HD (YAC128 mice). These effects were accompanied by increased phosphorylation of mutant huntingtin at Ser13 and Ser16, a post-translational modification that was shown to decrease mutant huntingtin toxicity. **Objectives.** The aim of this study was to further characterize the therapeutic effects of GM1 and to demonstrate that GM1 is a disease-modifying treatment for HD. **Methods.** We measured motor and non-motor behaviour in three different models of HD (R6/2, YAC128 and Q140 mice) during chronic intraventricular infusion of GM1 or vehicle. At the end of the treatment, filter-trap assay, immunoblotting and stereology analysis were performed to determine whether GM1 affects neurodegeneration and levels of mutant huntingtin aggregates in treated mouse brains. **Results.** GM1 treatment decreased levels of soluble and insoluble huntingtin in HD mouse brains, and dramatically improved both motor and non motor symptoms of the disease. In R6/2 mice, GM1 attenuated striatal neuron and white matter loss, increased body weight and

prolonged survival. **Conclusions**. Our data suggest that GM1 has disease-modifying properties across HD mouse models and could therefore have therapeutic potential in HD patients.

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Poster

418. Huntington's Disease Therapeutics

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Program#/Poster#: 418.05/X17

Topic: C.04. Movement Disorders

Title: Chronic Class IIa HDAC inhibition only partially replicates the beneficial effects of HDAC4 genetic reduction in HD models

Authors: O. AZIZ[1], C. A. LUCKHURST[1], T. HEIKKINEN[2], O. KONTKANEN[2], G. TOMBAUGH[4], S. GELMAN[5], D. YATES[1], K. MATTHEWS[1], R. WILLIAMS[1], P. BRECCIA[1], M. LAMERS[1], R. JARVIS[1], A. HAUGHAN[1], D. FISCHER[3], G. MCALLISTER[1], W. BLACKABY[1], A. GHAVAMI[4], G. OSBORNE[6], D. GOODWIN[6], G. BATES[6], I. MUNOZ-SANJUAN[7], C. DOMINGUEZ[7], L. PARK[7], M. MAILLARD[7], *V. BEAUMONT[7];
[1]Charles River, Saffron Walden, United Kingdom; [2]Charles River, Kuopio, Finland; [3]Charles River, Leiden, Netherlands; [4]Psychogenics Inc., Montvale, NJ; [5]Psychogenics Inc., montvale, NJ; [6]UCL Inst. of Neurol., London, United Kingdom; [7]CHDI Mgmt., Los Angeles, CA

Abstract: Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disorder resulting from a polyglutamine-encoding CAG expansion in the Huntingtin (*HTT*) gene. Targeting the Class IIa histone deacetylase HDAC4 appears to be a promising therapeutic strategy to treat HD. HDAC4 genetic reduction in the R6/2 HD mouse model ameliorated motor and CNS neurophysiological deficits and improved survival [1]. In addition, HDAC4 reduction delayed huntingtin (Htt) aggregation in CNS tissue from both the R6/2 and HdhQ150 full length KI mouse model of HD [1]. We have developed several chemical series of potent, selective, orally bioavailable and CNS-penetrant catalytic-site inhibitors of Class IIa HDACs [2,3], to probe whether HDAC4 inhibition can replicate the beneficial effects of HDAC4 reduction in HD models. This strategy is untested, as the deacetylase activity of Class IIa HDACs have been called into question [4]. Two structurally distinct lead series compounds have been evaluated in a battery of HD model interrogations, including R6/2 *in vivo* behavioral and survival endpoints and HD model *ex vivo* electrophysiological and molecular readouts following chronic dosing. These compounds; CHDI-00390576 and CHDI-00484077, differ as to the warhead used to engage the Zn^{2+} ion in the catalytic site of Class IIa HDACs (hydroxamate versus

trifluoromethyloxadiazole), their ability to disrupt HDAC4:HDAC3 associations, their pharmacokinetic (PK) and pharmacodynamic (PD) properties and their selectivity over Class I/IIb HDACs. We will present the detailed PK-PD biomarker and modeling approaches used to determine appropriate chronic dosing regimens for the inhibitors, and summarize our data where we determine that selective Class IIa catalytic site inhibition fails to replicate all beneficial effects seen following HDAC4 genetic reduction in HD models. While some neurophysiological deficits, restored following HDAC4 genetic reduction, are convincingly and dose-dependently restored in HD models following treatment with either compound; we failed to significantly impact behavioral deficits. We also had no effect on Htt aggregation levels through chronic dosing. Our results suggest that blocking the catalytic site of HDAC4 is insufficient to fully recapitulate the spectrum of biological functions that HDAC4 knock-down subserves in an HD context, whilst specifically improving some aspects of HD pathophysiology. 1] Mielcarek M., *et al.*, PLoS Biol, 2013. **11**(11): p. e1001717.[2] Burli R., *et al.*, J. Med Chem 2013. **56**(24): p9934-54[3] Luckhurst C.A., *et al.* ACS Med Chem Lett, 2016. **7**(1): p34-9 [4] Lahm A., *et al.* PNAS USA, 2007. **104**(44): p17335-40

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Poster

418. Huntington's Disease Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: C.04. Movement Disorders

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Ministerio de Ciencia e Innovación (SAF2012-39142 and SAF2015-67474-R)

Title: Dual therapeutic benefits of selective-histone deacetylase 3 inhibition in Huntington's disease mice

Authors: N. SUELVES[1,2,3,4], L. KIRKHAM-MCCARTHY[5,6], R. S. LAHUE[5,6], *S. GINES-PADROS[1,2,3,4];
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Abstract: Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by motor, cognitive and psychiatric symptomatology. Although motor features are the most evident cognitive deficits precede the overt motor symptoms by years in HD patients and are most highly associated with functional decline. Several studies have reported transcriptional dysregulation as an early underlying pathogenic mechanism in HD. Accordingly, therapeutic development has focused on targeting Histone deacetylases (HDACs) with small-molecule inhibitors in order to increase histone acetylation and raise gene transcription. However, the benefits of broad-based inhibitors in HD pathology could be counteracted by their potential toxicity, especially in chronic treatments. In this regard, Histone deacetylase 3 (HDAC3) has been implicated in HD mice as enforcing transcriptional aberrations and helps fuel expansions of CAG repeats in cultured cells. Importantly, it has also been reported that HDAC3 plays a critical role in memory formation.
In view of that, we have investigated whether RGFP966, an isotype-selective inhibitor of HDAC3, improves cognitive deficits and somatic CAG expansions in the $Hdh^{Q7/Q111}$ knock-in (KI) mouse model of HD. Behavioral assessment revealed that early chronic treatment with RGFP966 completely reversed altered motor learning and impaired recognition and spatial memories in KI mice. Biochemical and genetic analysis in mouse brain samples showed that systemic administration of RGFP966 normalized the expression of memory-related genes, partially recovered striatal pathological markers and decreased aggregation of mutant huntingtin. Using molecular biology techniques to assess expansion frequencies, we could detect that chronic treatment with RGFP966 also suppressed striatal CAG repeat expansions.
Taken together, our results reveal a good pharmacotherapeutic approach to prevent HD cognitive decline and, simultaneously, to suppress somatic CAG repeat expansions that drive disease progression.

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Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: NIH grantPN2EY016525

Larry L Hillblom Foundation

Title: TRiC subunits enhance BDNF axonal transport and rescue striatal atrophy in Huntington's disease

Authors: X. ZHAO[1], X.-Q. CHEN[1], E. HAN[1], Y. HU[1], P. PAIK[1], Z. DING[2], J. OVERMAN[3], A. LAU[3], S. SHAHMORADIAN[4], W. CHIU[4], L. THOMPSON[3], *W. C. MOBLEY[5], C. WU[1]; [1]UC San Diego, La Jolla, CA; [2]The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [3]Univ. of California, Irvine, Irvine, CA; [4]Baylor Col. of Med., Houston, TX; [5]Dept. of Neurosciences, Univ. of California San Diego Dept. of Neurosciences, La Jolla, CA

Abstract: Corticostriatal atrophy is a cardinal manifestation of Huntington's disease (HD). However, the mechanism(s) by which mutant Htt protein (mHtt) contributes to the degeneration of the corticostriatal circuits in HD is not completely understood. Herein, we investigated the mechanisms by which individual subunit of TRiC (TCP-1 Ring Complex or CCT) impacted the level of mHtt and striatal atrophy in HD. By recreating the corticostriatal neuronal circuit in microfluidic chambers using neurons from BACHD mice, we have demonstrated that CCT3 or purified apical domain of CCT1 (ApiCCT1) normalized BDNF transport from cortical to striatal neurons and rescued striatal neuronal atrophy in BACHD. CCT3 and ApiCCT1 also restored retrograde axonal transport of BDNF as well as lysosomal transport in cortical neurons. Further, both CCT3 and ApiCCT1 decreased the level of mHtt in BACHD cortical neurons and, in 14A2.6 cells. Our findings suggest that TRiC reagents promote selective degradation of mHtt to enhance supplies of cortical BDNF to striatal neurons.

Disclosures: X. Zhao: None. **X. Chen:** None. **E. Han:** None. **Y. Hu:** None. **P. Paik:** None. **Z. Ding:** None. **J. Overman:** None. **A. Lau:** None. **S. Shahmoradian:** None. **W. Chiu:** None. **L. Thompson:** None. **W.C. Mobley:** None. **C. Wu:** None.

Poster

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Support: National Institute of Health National Research Service Award Postdoctoral Fellowship (F32NS090722) (Fink)

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A Stewart's and Dake Family Gift(Fink)

Help4HD International

Title: An efficient antibiotic inducible gene therapy system for Huntington's Disease neurons

Authors: *A. KOMARLA[1,2], P. DENG[2,1], A. TORREST[2], J. APRILE[2], W. CARY[2], J. GUTIERREZ[2], W. GRUENLOH[2], G. ANNETT[2], T. TEMPKIN[3], V. WHEELOCK[3], D. J. SEGAL[1], J. NOLTA[2], K. FINK[2];
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Abstract: Huntington's disease (HD) is a genetic neurodegenerative disease that is caused by the abnormal expansion of the CAG repeats region the HTT gene on exon 1 of chromosome 4 that normally encodes for the huntingtin protein, known to facilitate neuron growth and maintenance. Accumulation of mutant huntingtin is toxic to neurons and causes the activation of pro-apoptotic molecules, eventually resulting in neuronal death. Selective loss of striatal neurons early in the disease results in symptoms such as chorea, cognitive and emotional disturbances and other forms of motor dysfunction.
Our lab uses Transcription Activator-Like Effectors (TALEs) partnered with Krüppel associated box (KRAB) domain to target Single Nucleotide Polymorphisms (SNPs) in mutant allele and cause selective transcriptional repression. Following delivery of TALEs into HD fibroblasts

using conventional transfection methods, we observed robust silencing of the mutant gene and subsequent reduction in mutant protein. However, transfection efficacy was limited but highly correlated to gene repression underlying the need for an optimized delivery platform.
The present study focused on a viral delivery method to increase transfection efficiency in combination with an antibiotic inducible system to allow regulated expression of the TALE-KRAB complex. We chose a TALE that demonstrated specific allele repression and targets a SNP that exists in a transgenic mouse model to clone into our Tet-On inducible lenti-TALE system (TTS). T3γ-KRAB was cloned into an inducible lentivirus with an eGFP reporter plasmid and the presence of the fluorescent reporter was analyzed using flow cytometry. We then tested the TTS in both primary fibroblasts isolated from HD patients and in primary neurons isolated from the YAC128 mouse model. Our primary outcome measures were the reduction of the mutant allele using SNP-genotyping and reduction of mutant huntingtin protein using Western Blot. This study will allow testing of a viral delivery method in partnership with an antibiotic inducible system that can provide useful information on future gene therapy delivery methods.

Disclosures: **A. Komarla:** None. **P. Deng:** None. **A. Torrest:** None. **J. Aprile:** None. **W. Cary:** None. **J. Gutierrez:** None. **W. Gruenloh:** None. **G. Annett:** None. **T. Tempkin:** None. **V. Wheelock:** None. **D.J. Segal:** None. **J. Nolta:** None. **K. Fink:** None.

Poster

418. Huntington's Disease Therapeutics

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 418.09/Y3

Topic: C.04. Movement Disorders

Support: CHDI A7449

Title: Behavioral and electrophysiological improvements following up-regulation of glt1 in the q175 Huntington’s mouse model

Authors: ***K. D. BUNNER**, C. RANGEL-BARAJAS, B. M. MCCORMICK, S. J. BARTON, G. V. REBEC;
Program in Neurosci. & Dept. Psychological & Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Huntington’s disease (HD) is an inherited neurodegenerative condition that typically strikes in mid-life with progressively deteriorating cognitive, emotional, and motor symptoms. While the sole cause of HD, a CAG repeat expansion on the short arm of chromosome 4, is known, currently no effective treatment or means to slow the progression of this disorder exists. Previous work by our group has shown electrophysiological and behavioral changes in the Q175

mouse that parallels changes seen in other HD mouse models. We found a significant decrease in quality of nest built and percentage of Nestlet used in both heterozygous (HET) and homozygous (HOM) Q175s compared to wild-type (WT) controls. Q175 mice also show a significant decrease in number of line crossings compared to WT mice along with changes in spontaneous behaviors (i.e. grooming, quiet rest, and rearing). In striatum, electrophysiological changes, including increased spike rate in HETs accompanied by a significant decrease in patterns of burst firing relative to WTs, suggest an early vulnerability to HD. Here, in light of inadequate glutamate uptake in both HD patients and mouse models, we increased expression of GLT1, the protein responsible for >90% of glutamate uptake, by viral-mediated gene transfer. Intravascular administration of adeno-associated virus serotype 9 (AAV9), which crosses the blood-brain barrier, was used to up-regulate GLT1. We assessed behavioral and striatal neuronal changes beginning at >6 weeks after AAV9-GLT1 injection. Although our data analysis is ongoing, we find that HET mice show patterns of both nest building and striatal neuronal activity that now parallel those of WT controls. Thus, our results, even at a relatively early stage of data analysis, support evidence for GLT1 as a therapeutic target for reversing the behavioral and neuronal deficits in HD.

Disclosures: K.D. Bunner: None. **C. Rangel-Barajas:** None. **B.M. McCormick:** None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

418. Huntington's Disease Therapeutics

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Program#/Poster#: 418.10/Y4

Topic: C.04. Movement Disorders

Support: CHDI A7449

Title: Effect of GLT-1 up-regulation on striatal local field potential activity and motor performance

Authors: *C. RANGEL BARAJAS, K. D. BUNNER, S. J. BARTON, G. V. REBEC; Psychological and Brain Sciences, Program in Neurosciences, Indiana Univ., Bloomington, IN

Abstract: Dysregulated glutamate transmission plays a key role in Huntington’s disease (HD), a dominantly inherited neurodegenerative condition caused by an expanded CAG repeat (Q) in the gene that encodes for the huntingtin protein (HTT). HD is characterized by progressively worsening cognitive, psychiatric, and motor symptoms. Although the search for an effective therapeutic target continues, it is noteworthy that HD patients as well as transgenic mouse

models of HD show a down-regulation of the glutamate transporter-1 (EAAT2 in humans and GLT1 in rodents). Here, we used the adeno-associated virus serotype-9 (AAV9) to induce viral-mediated gene transfer to increase GLT1 expression in the Q175 knock-in (KI) mouse model. We assessed rotarod performance in heterozygous (HET) and homozygous (HOM) Q175s as well as wild-type (WT) controls from ≈32-60 weeks of age. Striatal local field potential activity (LFP) was recorded from the same animals as they entered the choice point or center of a plus-shaped maze. The intravascular administration (0.1 ml, [$1x10^{13}$ vg/ml]) of AAV9-EAAT2 administered around ≈24 weeks of age, elevated the GLT1 expression in striatum, and significantly improved rotarod performance progressively by increasing the latency to fall at with a maximum performance at 50 weeks of age when compared with untreated mice (22.83 ± 4.6 s vs 43.05 ± 2.6 s), no significant effect was found in either wild type (WT) or homozygous (HOM) mice at the same age. In the plus-maze, the total number of arm choices was significantly increased in WT and HET especially at older ages (≈48 weeks of age), while in HOM mice the effect was mainly in the early stages (≈32-40 weeks of age). Although the turning probability in the plus maze was not affected by AAV9-EAAT2 treatment, the striatal LFP power spectral density for delta wavelength after the choice point on the maze, was increased in HET mice compared to untreated mice, while in HOM mice, the increased delta power observed in untreated mice was ameliorated by AAV9-EAAT2 administration. These data suggest that the increased expression of EAAT2 in might be a potential therapeutic treatment for the motor alterations observed in HD.

Disclosures: C. Rangel Barajas: None. **K.D. Bunner:** None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: NIA/NIH AG031153

NIA/NIH AG019206

Title: Molecular mechanism underlying defective BDNF secretion from astrocytes expressingmutant huntingtin

Authors: *Y. HONG, T. ZHAO, X.-J. LI, S. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Huntington's disease (HD) is a fatal, inherited, neurodegenerative disorder that affects one in every 10,000 Americans. However, there is no effective treatment for HD to date, in part because the pathogenic mechanism driving the disease is incompletely understood. A mutant form of the huntingtin protein (htt), in which a polyQ repeat region is greatly expanded, is a critical molecular feature of the disease. The huntingtin protein is necessary for multiple cellular functions, including gene transcription and vesicle trafficking. However, mutant htt is toxic to neurons. Most studies of neurodegenerative diseases have been focused on neurons because degeneration is observed mainly in neuronal cells. However, the survival of neuronal cells is also supported by glial cells such as astrocytes. One important role of astrocytes is to synthesize and release brain-derived neurotrophic factor (BDNF), which is vital for neuronal survival, development and function. Mutant htt is found in astrocytes both in the brains of HD patients and mouse models of the disease; however, little is known about the pathogenic role of mutant htt in astrocytes. In this study, we used a HD140Q knock-in mouse model and a transgenic HD mouse model (GFAP-160Q) that specifically expresses N-terminal mutant htt in astrocytes to study the effect of mutant htt on BDNF secretion. Our results indicate that compromised exocytosis of BDNF in HD astrocytes contributes to the decreased BDNF levels in HD brains and underscores the importance of improving glial function in the treatment of HD.

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Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: HDSA grant

Title: Immunoprecipitation and flow cytometry huntingtin lowering biomarkers

Authors: ***A. L. SOUTHWELL**[1], N. S. CARON[1], S. E. P. SMITH[2], Y. XIE[1], J. SONG[3], I. SEONG[4], B. R. LEAVITT[1], M. R. HAYDEN[1];
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Abstract: Huntington disease (HD) is a progressive neurodegenerative disorder caused by expansion of a polyglutamine encoding CAG trinucleotide repeat in the gene encoding the huntingtin protein (HTT). Although age of onset in HD can be predicted based on CAG repeat

length, this variable accounts for only 50-70% of the variation, while multiple known and unknown genetic and environmental factors account for the remainder. Thus, there is a need for biomarkers that could more accurately predict disease conversion or progression. Levels of mutant HTT protein or of specific species of mutant HTT protein in the brain could be such biomarkers. Additionally, there are multiple HTT lowering therapeutics in preclinical and clinical development, for which quantification of HTT levels in the brain is essential to validate and quantify target engagement. It is not currently possible to directly quantify HTT levels in the living brain of patients. To overcome this, we have developed an ultrasensitive mutant HTT detection method, micro-bead based immunoprecipitation and flow cytometry (IP-FCM), suitable for use in HD patient and model mouse cerebrospinal fluid (CSF). We have used IP-FCM to demonstrate that mutant HTT protein is elevated after disease onset in CSF from HD mutation carriers and that CSF mutant HTT level correlates to multiple clinical measures, including motor and cognitive performance. Using HD model mice, we have demonstrated that the brain is the major source of mutant HTT protein in CSF and that CSF mutant HTT protein levels reflect brain levels, decreasing following CNS HTT lowering intervention. Thus, mutant HTT IP-FCM has potential applications as a biomarker of HD onset/progression and as a target engagement and pharmacodynamic biomarker of HTT lowering therapies. In ongoing work we are using an allelic series of recombinant full-length huntingtin proteins (Q2, Q23, Q43, Q67, Q78) to further characterize mutant HTT IP-FCM. This will allow for more accurate comparison between individuals and for absolute protein quantitation. Additionally, we are continuing development of IP-FCM assays with different specificities, such as total HTT protein and cleaved HTT protein. These companion assays could be used in conjunction with mutant HTT IP-FCM to evaluate allelic effects of HTT lowering therapies or to investigate proteolytic processing of mutant HTT, respectively. These assays could provide essential clinical diagnostics and valuable research tools for studies of the pathogenesis of HD.

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Poster

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Topic: C.04. Movement Disorders

Support: Vaughan Foundation

NRF Grant 2011-0030049

Title: Proteasome activator, PA28γ, improves motor coordination and proteasome function in Huntington's disease YAC128 mice

Authors: *J. JANG[1], J. JEON[1], W. KIM[1], O. ISACSON[2], H. SEO[1];
[1]Hanyang Univ., Ansan, Korea, Republic of; [2]Neurodegeneration Inst., McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Huntington's disease (HD) is an autosomal dominant neurological disorder with progressive degeneration of medium sized spiny neurons (MSN) in the caudate putamen area. Previous studies showed the inhibited proteolytic activities of the ubiquitin–proteasome system (UPS) in HD patient's brain. It has been reported that proteasome activator (PA), PA28γ enhanced proteasome activities and cell survival in *in vitro* HD model. In this study, we found whether PA28γ gene transfer improves the proteasome activities and pathological symptoms in *in vivo* HD model. We stereotaxically injected lenti-PA28γ virus into the striatum of mutant YAC128 HD mice and littermate controls at 14–18 months of age, and validated their behavioral and biochemical changes at 12 weeks after the injection. YAC128 mice showed a significant increase in their peptidyl-glutamyl preferring hydrolytic (PGPH) proteasome activity and the mRNA or protein levels of brain derived neurotrophic factor (BDNF) and pro-BDNF after lenti-PA28γ injection. The number of ubiquitin-positive inclusion bodies was reduced in the striatum of YAC128 mice after lenti-PA28γ injection. YAC128 mice showed significant improvement of latency to fall on the rota-rod test after lenti-PA28γ injection. These data demonstrate that the gene therapy with PA28γ can improve UPS function as well as behavioral abnormalities in HD model mice.

Disclosures: **J. Jang:** None. **J. Jeon:** None. **W. Kim:** None. **O. Isacson:** None. **H. Seo:** None.

Poster

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Support: NIH NRSA F32 NS081964

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Hereditary Disease Foundation

CHDI Foundation, Inc.

Title: Bexarotene activation of PPAR-delta ameliorates preclinical trial outcomes in Huntington's Disease by promoting mitochondria metabolic function and autophagy

Authors: ***A. S. DICKEY**[1], D. SANCHEZ[1], N. LOMAS[1], K. R. SAMPAT[1], A. FLORES[1], W. FAN[2], N. ARBEZ[3], C. A. ROSS[3], R. M. EVANS[2], E. MASLIAH[1], A. R. LA SPADA[1]; [1]Sanford Consortium - UCSD, La Jolla, CA; [2]Salk Inst., La Jolla, CA; [3]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Huntington's disease (HD) is a relentlessly progressive autosomal dominant neurodegenerative disorder characterized by involuntary movements, cognitive decline, and psychiatric illness. Studies by our group and others have shown that mitochondrial dysfunction and metabolic deficits in HD result from transcriptional dysregulation of peroxisome proliferator-activated receptor [PPAR] gamma coactivator-1 alpha (PGC-1α). As a transcriptional regulator, PGC-1α modulates nuclear receptor transcription factors including PPARδ, the most abundantly expressed subtype in CNS. We recently reported that repression of PPARδ transactivation contributes to HD pathogenesis, and demonstrated in a rigorous preclinical trial that treatment of HD mice with the PPARδ agonist KD3010 is an effective therapy, significantly ameliorating motor phenotypes, markedly reducing striatal neurodegeneration, and greatly extending lifespan.

Another aspect of PPARδ biology with relevance for therapy development is that PPARδ forms a heterodimer with the retinoid X receptor (RXR), with availability of RXR as a limiting factor for PPARδ activity, so RXR agonists are capable of promoting PPARδ transactivation. One compound with potent RXR agonist activity is bexarotene, a synthetic compound structurally similar to retinoic acid, which upon administration to AD mice yielded dramatic improvements in behavioral deficits and enhanced clearance of Aβ oligomers. We tested if PPARδ activation may contribute to bexarotene neuroprotection in cell culture and primary neuron models of HD. Our findings indicate that bexarotene neuroprotection against htt protein neurotoxicity depends upon engagement with PPARδ.

To determine if bexarotene is a therapy candidate for HD, we performed a preclinical trial of bexarotene in HD N171-82Q mice, and documented significant improvements in motor phenotypes and neuropathology. We examined the mechanistic basis for PPARδ agonist neuroprotection by evaluating metabolic function in primary neurons from BAC-HD mice treated with KD3010 or bexarotene, and observed a dramatic rescue of impaired oxygen consumption, oxidative phosphorylation, and glycolytic activity upon KD3010 or bexarotene treatment. Also, these agonists rescued mitochondrial fragmentation, and increased autophagy. Our results provide strong support for PPARδ agonist therapy as a treatment for HD, uncover improved mitochondrial metabolic function as a central feature of PPARδ agonist neuroprotection, and highlight the PGC-1α - PPARδ pathway as an attractive therapy target for HD and related neurodegenerative disorders characterized by mitochondrial dysfunction.

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Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: J10130 PUCE

Title: Decreased of HSP 70, XIAP, ikbα, it15 genes expression and the CAG repeat expansion of animals treated with rnai in an experimental model of huntington's disease

Authors: *R. AVILES REYES[1], G. SARMIENTO[1], P. PALACIOS[2], S. ANDRADE[2];
[1]Univ. of Guayquil, Guayaquil, Ecuador; [2]Pontifical Catholic Univ., Quito, Ecuador

Abstract: The aggregation of the huntingtin protein (Htt) induces a progressive neurodegenerative disorder called Hunting Diease (HD). This pathology have an autosomal dominant inheritance. This neuropathology causes mutation of CAG trinucleotide repeat expansion. The CAG repeat is translated into a polyQ stretch. In this study we inoculated quinolinic acid (QA) intra cerebrally (Atlas of Paxinos and Watson, 1986: AP = +1.2 ahead of Bregma, L = +2.8 and DV = -5.5). Incisor bar location to 2 mm below the interaural line), to wistar rats in an experimental model of Huntington Disease. In the experimental design we worked with animal's treatment as: control; QA; QA+SS; QA+RNAi. In addition we analyzed HSP70, XIAP, IkBa, IT15 genes by RT-PCR. The sequencing technique was did only for IT15 gene. The result obtained of this research were found over expression of the genes analyzed. In the sequencing study we showed that the animals with RNAi had lower CAG repeat expansion. The results obtained evidenced a significantly RNAm expression in the QA and QA+SS animals, contrary that occurs in the animals with RNAi. It could support that: if we use an interference it could detain in some grade the fast progression of Huntington disease.
Keywords, Huntington Disease, RT-PCR, sequencing HSP70, XIAP, IkBa, RNAi, IT15, CAG repeat expansion

Disclosures: **R. Aviles Reyes:** A. Employment/Salary (full or part-time): University of Guayaquil. 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; Pontifical Catholic University, Quito Ecuador. **G. Sarmiento:** None. **P. Palacios:** None. **S. Andrade:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: NICHD Grant 1R01HD060630

Title: Functional brain reorganization after exercise training in the CAG140 knock-in mouse model of Huntington's disease

Authors: *D. P. HOLSCHNEIDER[1], Z. WANG[1], D. P. STEFANKO[2], W. A. TOY[2], Y. GUO[1], G. M. PETZINGER[2], M. W. JAKOWEC[2];
[1]Psychiatry & the Behavioral Sci., [2]Neurol., USC, Los Angeles, CA

Abstract: We recently reported genotypic differences in resting state cerebral perfusion in a Huntington's Disease (HD) mouse model characterized by a gene knock-in (KI) of a human exon 1 CAG140 expansion repeat (CAG140 KI mice). CAG140 KI animals showed significant hypoperfusion of the basal ganglia motor circuit, hyperperfusion of cerebellar-thalamic and somatosensory regions. These genotypic differences were noted in male mice at 6 months of age prior to the manifestation of motor deficits. We now report the effects daily treadmill exercise has on resting state perfusion maps following 5 months of daily treadmill training. Regional cerebral blood flow (rCBF) was mapped at rest in awake, nonrestrained, male, 6-month old CAG140 KI and wild-type (WT) mice using 14C-iodoantipyrine autoradiography. Results were analyzed in three-dimensionally reconstructed brains by statistical parametric mapping. Exercised compared to sedentary CAG140 KI mice showed an increase in rCBF of the dorsolateral striatum and posterior motor cortex (M1, M2), whereas exercise in WT mice demonstrated a decrease in rCBF. Exercised CAG140 KI mice compared to exercised WT animals showed increased rCBF in the cerebellum, the ventrolateral thalamus and posterior-most motorsensory cortex, suggesting a greater engagement of the cerebellar-thalamocortical pathway. Our results suggest that exercise elicits a functional reorganization of whole-brain networks at a presymptomatic stage in the life of the CAG140 KI mouse.

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Poster

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Topic: C.04. Movement Disorders

Support: CHDI Grant to JF Cheer

Title: Pharmacological elevation of 2-arachidonoylglycerol brain levels rescues motivational dysfunction and accumbal correlates in a Q175 mouse model of Huntington's disease

Authors: *H. M. DANTRASSY[1], D. P. COVEY[1], N. E. ZLEBNIK[1], H. QADIR[1], E. COLE[1], M. A. ANAYA[1], I. GILDISH[1], J. F. CHEER[1,2];
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Abstract: Huntington's disease (HD) is an inherited neurodegenerative disorder caused by a polyglutamine (CAG) expansion in the huntingtin gene. Striatal and cortical dysfunction are the hallmark neuropathological features underlying dyskinesia (e.g., chorea) and akinesia at later stages. However, prominent psychiatric symptoms manifest prior to motor dysfunction. We have recently shown in the Q175 mouse model of HD that suppressed motivation - one of the earliest indicators of HD - is associated with compromised dopaminergic signaling and network dynamics in the nucleus accumbens (NAc). Because NAc functioning and motivation are tightly regulated by cannabinoid type 1 receptor (CB1R) function, and CB1R expression and function are disrupted in HD, we assessed CB1R regulation of NAc activity and motivation. To characterize this relationship, *in vivo* electrochemical and electrophysiological recordings were collected in the NAc of wild type (WT) and Q175 knock-in mice. Separate groups of animals were chronically implanted with carbon fiber microelectrodes to measure subsecond dopamine release or multi-electrode arrays to record extracellular local field potentials and single-unit cell firing. Recordings were obtained during a progressive ratio (PR) schedule of reinforcement following treatment with the monoacylglycerol lipase inhibitor JZL-184 and the CB1R inverse agonist AM-251. Relative to WT controls, raising tissue levels of the endocannabinoid (eCB) 2-arachidonoylglycerol (2-AG) in HD mice rescues deficits in NAc dopaminergic and single-unit encoding of reward, attenuates abnormal gamma power and ameliorates motivational deficits. Our findings indicate that modulating NAc abnormalities with eCB-based therapies may be beneficial in treating the prodromal psychiatric deficits in HD.

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Poster

418. Huntington's Disease Therapeutics

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Support: Zumberge Foundation

USC Provost Collaboration Grant

NIH NICHD 1R01HD060630

Title: Exercise-enhanced neuroplasticity modifies disease progression in the CAG_{140} knock-in mouse model of Huntington's disease

Authors: *M. W. JAKOWEC[1], D. STEFANKO[1], Z. WANG[2], W. TOY[1], D. HOLSCHNEIDER[2], G. PETZINGER[1];
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Abstract: Epidemiological studies support that physical activity plays an important role in providing protection fromthe neurorehabilitation of a number of neurological disorders, including Parkinson's disease, Alzheimer's disease, and age-related cognitive impairment. In animal models of these disorders, physical activity in the form of exercise (treadmill running) can restore both motor and cognitive behavioral deficits and modify disease progression. While some studies have begun to address the role of physical activity in rodent models of Huntington's disease (HD) we Our laboratory has been are interested in exploring the impact of exercise as a long-term component of lifestyle in a model of Huntington's disease (HD)HD with a long prodromal period. For these studies, we subjected CAG_{140} knock-in mice to running on a motorized treadmill starting at 1 month of age, 3 days per week for 1 hour, and continuing for 1 year. This line demonstrates a long prodromal period and does not display HD-like motor symptoms until after 12 months of age. However, starting at 4 months of age, HD mice displayed cognitive deficits and depression-like behaviors, as well as the formation of aggregates of the mutant huntingtin mHtt protein (mHtt)aggregates. We found that exercise alleviates alleviated non-motorcognitive deficits and depressive behaviors and significantly reduces reduced HD pathology. We also examined the impact of exercise on dendritic spine density and expression of proteins involved in synaptic integrity, which are were altered by exercise in this model. At 12 months of age, with continuing exercise, there is was a delay in the onset of motor symptoms in HD mice compared to sedentary mice. To better understand the relationship between exercise and its neural substrates, we have begun to explore functional brain mapping changes with perfusion autoradiography using the tracer [^{14}C]-iodoantipyrine. Interestingly, sedentary CAG_{140} KI mice at 6 months of age showed hypo-perfusion of the basal ganglia circuit and hyper-

perfusion of several regions, including cerebellar-thalamic and somatosensory regions, indicating significant early cerebral functional re-organization in this model. Taken together, these studies begin to demonstrate that long-term changes in lifestyle, including physical activity has ave significant impact on brain health and may offer insight into mechanisms that may serve as targets in discovering novel therapeutic modalities to treat brain disorders such as HD.

Disclosures: M.W. Jakowec: None. **D. Stefanko:** None. **Z. Wang:** None. **W. Toy:** None. **D. Holschneider:** None. **G. Petzinger:** None.

Poster

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Support: DST-SERB, New Delhi SB/FT/LS-139/2012

Title: L-theanine, a component of green tea spares striatal neurons from 3-NP induced neurotoxicity: Role of Nitric Oxide pathway

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Abstract: Aim and Background: L-theanine is unique amino acid which readily crosses blood brain barrier and posses neuroprotective potential against neurodegenerative disorders including Huntington Disease (HD). HD is characterized by selective loss of GABAergic medium spiny neurons. Therefore, the present study was intended to investigate the effect of L-theanine against 3-NP induced striatal toxicity. **Methods:** Rats were administered with 3-NP for 21 days. L-theanine was given once a day, 1 hour prior to 3-NP treatment for 21 days and L-NAME (NOS inhibitor) and L-arginine (NOS activator) were administered 1 hour prior to L-theanine treatment. Body weight, behavioral parameters (locomotor activity, grip strength, narrow beam walk) observation was done on weekly basis up to 3 weeks after 3-NP treatment. On 22^{nd} day, animals were sacrificed and rat striatum was isolated for biochemical (mitochondrial complex-II, LPO, GSH and nitrite), pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and neurochemical analysis (GABA, Glutamate, DA, NE, 5-HT, DOPAC, HVA, 5-HIAA and Adenosine). **Results:** 3-NP treatment significantly altered body weight, locomotor activity, motor coordination, mitochondrial complex-II activity, oxidative defence, pro-inflammatory mediators (TNF-α, IL-6 and IL-1β), and striatal neurotransmitters level i.e. GABA, glutamate, catecholamines, adenosine, inosine and hypoxanthine whereas L-theanine treatment (25 & 50

mg/kg/day, p.o.) significantly prevented these alterations. Concurrent treatment of L-NAME with L-theanine (25 mg/kg/day, p.o.) significantly enhanced protective effect of L-theanine (25 mg/kg/day, p.o.) whereas concurrent treatment of L-arginine with L-theanine (50 mg/kg/day, p.o.) significantly decreased the protective effect of L-theanine (50 mg/kg/day, p.o.). **Conclusion:** The neuroprotective potential of L-theanine involves inhibition of detrimental nitric oxide production and prevention of neurotransmitters alteration in striatum.

Disclosures: S. Jamwal: None. **P. Kumar:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: NIH NINDS R01 NS079450

Title: Effect of amyloid precursor protein knock-down in the YAC128 mouse model of Huntington's disease.

Authors: K. L. BERGGREN[1], S. AGRAWAL[1], J. A. FOX[1], R. NELSON[2], *J. H. FOX[1]; [1]Vet. Sci., [2]Zoology-Physiology, Univ. of Wyoming, Laramie, WY

Abstract: Huntington's disease (HD) is an autosomal dominant disorder caused by a CAG expansion in the *HTT* gene. Elevated brain iron in susceptible brain regions is a consistent feature of murine and human HD. Amyloid precursor protein (APP) is implicated in neuronal iron export and levels are decreased in the R6/2 mouse HD model. Here we tested the effect of genetic knock down of APP on disease progression in YAC128 mice. APP hemizygous mice ($APP^{+/-}$) were crossed with YAC128 transgenic HD mice to generate $APP^{+/+}$ $HD^{+/-}$, $APP^{+/-}$ $HD^{+/-}$, $APP^{+/+}$ $HD^{-/-}$ and $APP^{+/-}$ $HD^{-/-}$ mice. Behavioral, brain biochemical and morphometric outcomes were determined. Mutant huntingtin protein (mhtt) expression resulted in an age-dependent decrease in rota-rod motor endurance. $APP^{+/-}$ mice also had decreased motor endurance as compared to $APP^{+/+}$ mice. Nest building behavior over a 24-hour period was also assessed. Both HD and $APP^{+/-}$ mice had significantly lower nest building scores and there was also a significant HD x APP interaction (p=0.0065). Stereologic analysis at 12-months of age revealed that YAC128 HD mice had smaller striatal volumes and fewer striatal neurons (p=0.0424) but there was no effect of APP genotype. Both cortical and striatal iron levels were significantly increased in $APP^{+/-}$ $HD^{+/-}$ as compared to $APP^{+/+}$ $HD^{+/-}$ mice (p-values=0.0155 and 0.0202, respectively); however, there were no effects of APP knock down on striatal and cortical iron levels in wild-

type mice. The findings indicate that YAC128 mice are more sensitive to the effects of genetically decreased APP on brain iron as compared to wild-type mice. APP knockdown potentiates some markers of HD progression.

Disclosures: K.L. Berggren: None. **S. Agrawal:** None. **J.A. Fox:** None. **R. Nelson:** None. **J.H. Fox:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: NIH NRSA

NIH Nanomedicine Program-CPFM

Title: Therapeutic delivery strategies for the apical domain of CCT1 in Huntington's disease.

Authors: *J. OVERMAN[1,2], Z. CROOK[3], Z. TAN[2], A. LAU[2], L. JOACHIMIAK[4], E. SONTAG[4], A. TOMLINSON[4], J. REIDLING[2], B. DEVERMAN[5], C. GLABE[2], J. FRYDMAN[4], D. HOUSMAN[3], L. THOMPSON[2];
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Abstract: Huntington’s disease (HD) is associated wtih aggregation of misfolded mutant Huntingtin (mHTT), ultimately resulting in intranuclear and cytoplasmic inclusions within neurons and neurite processes. While the precise role of aggregation in HD is not clear, protein misfolding and the aggregation process appears relevant to aberrant mHTT accumulation. The chaperone protein CCT (chaperonin containing TCP-1/TCP-1 ring) binds and folds proteins during *de novo* protein synthesis. Our previous work demonstrated that exogenous delivery of the substrate-binding apical domain of subunit 1 of CCT (ApiCCT1) is sufficient to decrease mHTT aggregation and rescue mHTT-mediated toxicity in multiple cell models of HD, making ApiCCT1 a promising therapeutic for HD. We find that recombinant ApiCCT1 ($ApiCCT1_r$), when exogenously delivered to cells, is able to enter both PC12 cells and primary cortical neurons (PCNs) and localize to the nucleus. Current studies focus on developing approaches to deliver $ApiCCT1_r$ to the brain, taking advantage of the ability of ApiCCT1 to penetrate cell membranes. We are investigating the use of both neural stem cells and viral vectors to deliver a secreted form of ApiCCT1 into the striatum of HD mice to provide continuous delivery of

ApiCCT1. Preliminary *in vivo* data suggests that direct injection of purified ApiCCT1$_r$ and mNSCs engineered to secrete ApiCCT1 (sApiCCT1) significantly reduce oligomeric mHTT following striatal transplantation in HD mice. Additional pilot studies indicate that R6/1 mice given intrastriatal injection of AAV2/1-sApiCCT1 show reduced oligomeric mHTT, fewer cells containing inclusion bodies, and behavioral improvements. Experiments are ongoing to test additional viral vectors. Preliminary data and published results suggest that *in vivo* delivery of secreted ApiCCT1 may provide an effective mechanism to modulate mHTT accumulation in HD.

Disclosures: J. Overman: None. **Z. Crook:** None. **Z. Tan:** None. **A. Lau:** None. **L. Joachimiak:** None. **E. Sontag:** None. **A. Tomlinson:** None. **J. Reidling:** None. **B. Deverman:** None. **C. Glabe:** None. **J. Frydman:** None. **D. Housman:** None. **L. Thompson:** None.

Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Title: Effects of PARP-1 inhibition on CREB-binding protein in the striatal neuronal subpopulations of the R6/2 mouse model of Huntington's disease

Authors: *F. R. FUSCO[1], E. PALDINO[1], A. CARDINALE[1], I. SAUVE[1], V. D'ANGELO[2], C. GIAMPÀ[3];
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Abstract: Poly(ADP-ribose) polymerases (PARPs) are NAD-dependent enzymes able to catalyse the transfer of ADP-ribose units from NAD to substrate proteins. PARP-1 inhibitors have been studied because they reduce post-ischaemic and excitotoxic neuronal death. In fact, we have showed that INO-1001, a PARP 1 inhibitor, displays a neuroprotective effect in the R6/2 model of HD. In this study, we investigated the effects of PARP1-inhibition induced modulation of pCREB on CBP localization in the striatal neurons, and on the degeneration of parvalbuminergic interneurons, which are a particularly vulnerable subclass of striatal neurons. Transgenic mice were treated with INO 1001 20 mg/Kg daily starting from 4 weeks of age. Results: We found that INO-1001 treated R6/2 mice. Double-label immunofluorescence was performed to value the distribution of CBP in ubiquitinated NIIs in the striatum. INO-1001-treated and saline-treated brain sections were incubated with: goat anti-choline acetyl transferase (ChAT; Nova Biological); goat anti-nitric oxide synthase (NOS; Sigma, St-Louis, MO); mouse

anti-parvalbuminergic (PARV, chemicon) and mouse anti-calretinin (CALR; Chemicon).Morphometric evaluation and cell counts were performed. Our study showed that INO-1001 has a positive effect in sparing parvalbumin interneurons of the striatum, where CREB is upregulated by the compound. Moreover, CBP localization is positively influenced by INO-1001, where intraneuronal levels are reinstated in the R6/2 mouse. The sum of our data corroborates the previous observations indicating PARP inhibition as a possible therapeutic tool to fight Huntington's disease

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Poster

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Support: National University of Singapore

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Teva Pharmaceutical

Title: Pridopidine treatment improves motor and psychiatric-like phenotypes in the YAC128 mouse model of Huntington disease

Authors: *M. GARCIA-MIRALLES[1], L. TAN[2], M. LIM[2], A. ORBACH[3], M. GEVA[3], M. HAYDEN[4], M. POULADI[2];
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Abstract: Pridopidine is currently in clinical development for the treatment of Huntington disease (HD) and investigations to increase the understanding of its therapeutic benefit and mode of action are ongoing. Here we aim to investigate the efficacy and mechanism of action of pridopidine using the transgenic YAC128 mouse model of HD. Pridopidine was administered to animals starting at early (1.5 months of age) or late stages of disease (8 months of age). In the early treatment cohort, animals were divided into three groups receiving 0, 10, or 30 mg/kg of pridopidine for a period of 10.5 months. In the late cohort, animals were divided into two groups

receiving either 0 mg/kg or an escalating dose of pridopidine (10 mg/kg in week 1, 20 mg/kg in week 2, and 30 mg/kg in weeks 3-8). Pridopidine treated animals were evaluated using a battery of behavioural tests. Our analysis reveals that chronic treatment with pridopidine improves behavioural measures including motor learning, motor performance and depressive phenotypes in the YAC128 HD mice. Specifically, pridopidine improved motor learning in the rotarod test, and motor performance in the accelerating rotarod and climbing tests, immobility in the forced swim test of depression, and decreased anxiety-like behaviour in the open field test. Follow-up studies currently underway are examining the effect of acute pridopidine treatment on motor and depressive phenotypes. Ongoing analyses to address the impact of pridopidine treatment on neuropathological measures include structural MRI, immunohistochemical and stereological assessments of striatal regions. Finally, molecular studies examining putative targets of pridopidine activity will shed light on its mode of action in the YAC128 HD mice. Overall, our study supports continued clinical development of pridopidine for HD.

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Poster

418. Huntington's Disease Therapeutics

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Title: Comparison of delivery modalities for *In vivo* administration of transcription activator like effectors in a Huntington’s disease model

Authors: *P. DENG[1], A. E. KOMARLA[1], A. M. TORREST[2], J. A. APRILE[2], J. GUTIERREZ[2], G. M. ANNETT[2], D. J. SEGAL[1], J. A. NOLTA[2], K. D. FINK[2];
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expansion of CAG repeats in the HD gene. Reduction of mutant huntingtin (muHtt) through protein interference or conditional gene knockout could prove to be an effective therapy. Previous work established allele-specific targeting and silencing by designing transcription activator-like effectors (TALE) to target single-nucleotide polymorphisms (SNP) in the mutant allele with a KRAB to promote transcriptional repression. We developed multiple TALEs that significantly reduce muHtt at the RNA and protein level without affecting the healthy allele in HD fibroblasts. Up to a 75% reduction of the mutant allele was observed following purification of only cells that received the TALE. The current study focuses on the examination of our lead TALE, T3γ, in a panel of adult and juvenile HD fibroblasts, primary YAC128 cortical and striatal neurons, and following injection in the YAC128 transgenic mouse model of HD. T3y was selected as our lead candidate TALE based on high global minor allele frequency score, robust gene silencing, allele-specificity, and the presence of its target SNP in the YAC128 HD mouse. Mutant allele knockdown was observed across multiple adult and juvenile HD fibroblasts following T3y treatment, demonstrating consistent gene silencing.
Preliminary data has shown robust expression of T3y-KRAB following in-vivo transfection using plasmid DNA complexed with a cationic polymer, TurboFect. However, limited biodistribution was observed. Our *in vitro* data indicates that wider coverage of target tissue with our TALE is vital for effective gene silencing. Presently, we compared the delivery of T3y-KRAB with: Invivofectamine (IVV), adeno-associated virus (AAV), and lipid nanoparticles (LNP) while using TF as our baseline control. IVV is a lipid-based RNA-delivery vesicle that has shown high *in vivo* transfection efficiency and gene silencing following *in vivo* injection in mice. AAV has been well tolerated in a number of clinical trials for neurodegenerative diseases and has demonstrated strong local cell penetrance. Finally, LNPs have shown to have strong potential as a delivery vehicle for biopharmaceuticals by complexing with ApoE secreted by astrocytes, allowing it to be uptaken by neurons and glia with minimal in-vivo toxicity and immunogenicity. We compared transgene expression, biodistribution, toxicity, and muHtt silencing following striatal injection of T3y-KRAB in the YAC128xNSG mouse model. Identification of both a potent and widespread delivery vehicle will be necessary to advance our target therapy to long-term functional efficacy studies.

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Poster

418. Huntington's Disease Therapeutics

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Strategic Positioning Fund for Genetic Orphan Diseases (SPF2012/005)

Title: The effects of environmental enrichment on myelination and oligodendroglia in the YAC128 model of Huntington's disease

Authors: *C. I. RADULESCU, M. GARCIA-MIRALLES, C. FERRARI BARDILE, M. A. POULADI;
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Abstract: An enriched environment (EE) is comprised of conditions that facilitate sensory, motor, cognitive and social stimulation, along with access to novelty and complexity. In animal studies, enrichment has been shown to improve, or delay, motor and cognitive deficits in several neurological and neurodegenerative disorders. In contrast, social isolation has been revealed to contribute to cognitive decline, and have negative effects on development and aging. Furthermore, robust evidence supports an association between behavioural experience and neurogenesis, and increasingly a link between environmental conditions, oligodendrogenesis and myelination is becoming apparent. White matter abnormalities have recently been exposed in the early stages of Huntington disease (HD), an autosomal dominant neurodegenerative disorder characterised by motor, cognitive and psychiatric disturbances. In the current study we investigated the impact of environmental enrichment on HD-related phenotypes in the YAC128 mouse model, and in particular the extent to which the status of the environment could modulate myelination and oligodendroglia. Animals of mixed gender and genotype (wild type and YAC128) were allocated to one of three conditions: (a) EE, (b) standard housing, or (c) socially isolated and deprived housing. Enrichment involved larger than standard housing, increased social contact, complex nesting, appropriate sheltering, and the inclusion of objects of various size, shape and texture. Animals were kept in their assigned condition for 12 weeks prior to testing. Mice were evaluated using tests of motor, anxiety and depressive behaviour over the course of two weeks to examine the impact of enrichment or social isolation on behavioural HD phenotypes. On-going assessments include behavioural analysis, transcriptional analysis of myelin-related genes, ultrastructural characterization of myelin sheaths using electron microscopy imaging, and stereological analysis of oligodendroglia cellular populations. Our

results on the effects of EE and social isolation on myelination and oligodendroglia, in both the HD and the healthy brain, may have important therapeutic implications for diseases involving white matter pathology such as HD.

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Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

Support: PhD funded by the Brain Research Trust

Title: Association between abnormal interhemispheric information transfer and corpus callosal structure in premanifest Huntington's disease

Authors: *H. E. CRAWFORD[1], A. MULICK CASSIDY[2], S. J. TABRIZI[1], R. I. SCAHILL[1]; [1]Huntington's Dis. Res. Centre, UCL, London, United Kingdom; [2]Dept. of Med. Statistics, London Sch. of Hyg. & Tropical Med., London, United Kingdom

Abstract: Recent research has found evidence of significant corpus callosal atrophy in Huntington's disease (HD), even many years prior to disease onset. The corpus callosum (CC) plays a significant role in interhemispheric information transfer (IIT) and is involved in many cognitive processes by way of its connectivity to numerous brain areas. The present study aimed to better understand how known degeneration of the CC in HD affects IIT, particularly in the premanifest stage, as well as investigate the association between IIT and CC volume and microstructural integrity.

An IIT task was administered to 12 premanifest HD and 14 healthy control subjects at two time points, one year apart. This very simple, unimanual reaction time (RT) task was designed to measure the transfer of information across the CC by exploiting the process of how stimuli presented to the left or right visual field is projected to the contralateral hemisphere. Linear regression models tested for within- and between-group differences in RT due to hand dominance and the crossing of stimulus presentation and hand response, as well as the between-group difference in IIT RTs. Regression models also examined the association between IIT and CC volume, as well as between IIT and the microstructural integrity of CC tracts, measured using 3T MRI and DTI, data which was collected previously as part of the TrackOn-HD study. Preliminary analysis of the cross-sectional behavioural results revealed that despite premanifest

HD subjects demonstrating overall slower RTs, there was no evidence of a difference in IIT RT between the groups. The groups however did demonstrate a reversal of IIT RTs. A larger crossed-uncrossed difference (CUD) for the non-dominant hand in healthy controls suggests that IIT takes longer to transfer from the dominant to the non-dominant hemisphere, whereas a larger dominant hand CUD in premanifest HD subjects suggests that IIT actually takes longer in the opposite direction.
A negative association between CC volume and fractional anisotropy (FA) in the genu tracts of the CC and non-dominant hand IIT was found in premanifest HD subjects. This suggests that as CC volume and FA decreases, the time it takes for information to cross between hemispheres, specifically from the dominant to the non-dominant hemisphere, increases.
In conclusion there is clearly an abnormality in premanifest HD subjects' IIT compared with controls, specifically when using the non-dominant hand but whether this is due to premanifest HD subjects being slower to respond overall is yet to be determined. Further analysis is needed to ascertain the exact patterns of IIT and to better elucidate the link between CC structure and function.

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Poster

418. Huntington's Disease Therapeutics

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Topic: C.04. Movement Disorders

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Title: Dynamic functional network connectivity differences between prodromal Huntington's disease & healthy control subjects

Authors: F. A. ESPINOZA[1], R. MILLER[1], E. MENNIGEN[1], V. M. VERGARA[1], J. A. TURNER[2], M. MISIURA[2], J. CIAROCHI[2], H. JOHNSON[3], J. D. LONG[3], J. BOCKHOLT[3], J. S. PAULSEN[3], *V. CALHOUN[1];
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Abstract: Background: Huntington's disease (HD) is a neurodegenerative disorder caused by a cytosine-adenine-guanine (CAG) expansion ≥36 in the HTT gene. HD affects motor coordination, leads to mental decline, and presents behavioral symptoms. Subtle changes in

motor and cognitive skill can be detected decades prior to receiving a diagnosis of HD. This time epoch is typically referred to as the prodrome of HD. Previous structural brain studies have shown that HD progress is characterized by striatal atrophy and cortical changes. In this study, we used resting-state functional magnetic resonance imaging (rsfMRI) to explore time-varying connectivity differences between individuals in the HD prodrome (prHD) and healthy control (HC) subjects by group independent component analysis (ICA).
Methods: We selected a sample of 261 subjects (183 prHD and 78 HC) from the PREDICT-HD study. Group ICA was used to decompose the rsfMRI data into 100 maximally spatially independent components (ICs) and associated time-courses (TCs), of which 46 ICs were identified as meaningful resting-state networks (RSNs) and were categorized into 8 functional domains. Using the sliding windows method, 106 dynamic functional network connectivity (FNC) matrices were estimated per subject as the pairwise correlations among the 46 RSN TCs. A k-means algorithm was used to assign FNC matrices into 4 clusters representing discrete states. Finally, multiple regression analysis with age, gender, CAG, and motion regressors was applied to each FNC state.
Results: The CAG variable and univariate test were used to identify group differences (significance was assessed at $p<0.05$ and FDR corrected). Only states 3 and 4 showed significant group differences. State 4 resembles the static FNC matrix including a negative correlation between CAG and the connectivity in the visual network. In addition, negative correlations between CAG and the interactions among subcortical network and default mode and salience networks; auditory and default mode, are observed only in the dynamic analysis. In state 3, we see multiple negative effects of CAG on connectivity to the putamen and thalamus involving auditory, cerebellar, cognitive control, default mode, salience, sensorimotor. Controls also spend significantly more time in state 3 compared to pro-HD.
Conclusions: Findings show significant dynamic functional connectivity differences between prHD and HC subjects in specific networks consistent with previous static analyses. In sum, prHD exhibit substantial dynamic connectivity differences relative to controls in rsfMRI data suggesting transient disruptions of connectivity may be a hallmark of the disease.

Disclosures: **F.A. Espinoza:** None. **R. Miller:** None. **E. Mennigen:** None. **V.M. Vergara:** None. **J.A. Turner:** None. **M. Misiura:** None. **J. Ciarochi:** None. **H. Johnson:** None. **J.D. Long:** None. **J. Bockholt:** None. **J.S. Paulsen:** None. **V. Calhoun:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.01/Z3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grant-in-Aid for Scientific Research from Japan Society for the promotion of Science

Grant for Project Research from Fukushima Medical University

Title: Phosphorylation of respiratory chain components by mitochondrial c-Src is required for neuronal viability

Authors: *M. OGURA, J. YAMAKI, M. K. HOMMA, Y. HOMMA;
Dept. of Biomolecular Sci., Fukushima Med. Univ. Sch. of Med., Fukushima, Japan

Abstract: Reversible protein phosphorylation is emerging as an important mechanism to modulate mitochondrial functions. Expression level of non-receptor-type tyrosine kinase c-Src is relatively high in mitochondria. The Src family kinase inhibitor amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo [3,4*d*] pyrimidine (PP2) exhibits significant reduction of respiration and production of reactive oxygen species (ROS). Similar results were obtained from cells expressing kinase-dead c-Src, which harbors a mitochondrial-targeting sequence. These results suggest that c-Src is involved in mitochondrial energy production and cell survival. In this study, we have identified novel substrates of c-Src in mitochondria and investigated their function in the regulation of oxidative phosphorylation and cell viability. Phosphorylation-site analysis selects c-Src targets, including NADH dehydrogenase [ubiquinone] flavoprotein 2 (NDUFV2) at Tyr193 of respiratory complex I and succinate dehydrogenase A (SDHA) at Tyr215 of complex II. Comparison of cells expressing wild-type and their phosphorylation-defective mutants reveals that NDUFV2 phosphorylation is required for NADH dehydrogenase activity and ATP production. SDHA phosphorylation is not required for enzyme activity, but required for efficient electron transfer. Expression of its phosphorylation-defective mutant induces significant amounts of ROS. Loss of viability is observed in the primary neurons expressing these mutants. The cleavage of poly (ADP-ribose) polymerase-1 is also observed in cells expressing NDUFV2 mutant, but not SDHA mutant, suggesting that NDUFV2 phosphorylation is required for prevention of apoptosis. These results suggest that mitochondrial c-Src regulates the oxidative phosphorylation by phosphorylating respiratory components and cell survival.

Disclosures: **M. Ogura:** None. **J. Yamaki:** None. **M.K. Homma:** None. **Y. Homma:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.02/Z4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH 5P20MD006988

Title: Docosahexaenoic acid (DHA) protects Schwann cells against palmitic acid-induced lipotoxicity through P38 but not JNK MAPK

Authors: *M. DESCORBETH[1], M. DE LEON[2];
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Abstract: Saturated fatty acids induce cell and tissue damage in the nervous system, whereas docosahexaenoic acid (DHA), an n-3 polyunsaturated fatty acid, shows a neuroprotective effect. The mechanism by which DHA protects the nervous system is unclear. In this study, we examined the contribution of the P38 and JNK MAPK pathways in the protective effect of DHA under palmitic acid-induced lipotoxicity (PA-LTx). We cultured primary Schwann cells in euglycemic (EG) (5.5 mM glucose) and hyperglycemic (HG) (17 mM glucose) conditions for 5 days and then treated them with PA: BSA (300 μM: 150 μM) in the presence or absence of DHA (50 μM) for up to 48 hrs. We used selected MAPK inhibitors to investigate the implication of P38 and JNK pathways in the DHA protection of pSC from PA-LTx. We showed that PA induced SC death, and that was associated with an increase in the phosphorylation of P38 and JNK in EG and HG groups. The addition of SB, a P38 inhibitor, or JNK II, a JNK inhibitor, with PA did not prevent PA to induce cells death. However, the combination of both inhibitors in presence of PA restored the cell viability in both groups. When we co-treated Schwann cell with DHA and PA the cell viability was also restored. In addition the chromatin condensation, an apoptosis feature, induced by PA was eliminated in the presence of DHA. Furthermore, DHA also restored P38 but not JNK phosphorylation to the control levels. In conclusion, we show that PA induced cell death through the P38 and JNK pathways and that DHA protects Schwann cells against PA by regulating P38 but not the JNK pathway.

Disclosures: M. Descorbeth: None. **M. De Leon:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.03/Z5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grants NS67078 (PZ), NS34179 (CI),

Title: Cysteine s-nitrosylation of neuroprotective protein prohibitin modulates mitochondrial dynamics in neurons

Authors: L. QIAN, Y. QU, G. MANFREDI, C. IADECOLA, *P. ZHOU;
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Abstract: The neuroprotective mitochondrial protein Prohibitin (PHB), is upregulated in a nitric oxide (NO) dependent manner upon ischemic preconditioning induction. However, the mechanisms of NO modulation of PHB remain to be elucidated. We had previously determined that PHB is nitrosylated at its only cysteine residue (Cys^{69}). Here, we focused on the effects of PHB nitrosylation on mitochondrial fission and fusion, the fundamental processes by which the imbalance result in cell degeneration (Chen and Chan 2010). We hypothesized that mitochondrial dynamics could be altered, if Cys^{69} were mutated to eliminate PHB nitrosylation. We tested this hypothesis by first transfecting neurons with PHB siRNA to downregulate endogenous PHB and then co-transfecting them with mt-dsRed to visualize mitochondria and C69V-PHB, a mutant DNA construct, in which the Cys^{69} is changed to Val. Five days later, live cell imaging of mt-dsRed was captured. Neurons were divided into three categories based on their mitochondrial morphology in neurites: neurons with normal elongated, with fragmented, or a mixture of normal and fragmented, mitochondria. The majority of neurons transfected with mt-dsRed only (control) had elongated mitochondria (65.6±3 % vs. 17.6±3% fragmented). Transfection with siRNA alone resulted in endogenous PHB decrease (50%) associated with an increase of neurons with fragmented mitochondria (30.7±3 % elongated vs 44.1±2 fragmented). Transfection of PHB silenced neurons with WT-PHB largely restored mitochondrial morphology to control levels (68.8±4% elongated vs 14.5±3 fragmented), while C69V-PHB failed to do so (31±1 elongated vs 42.2 fragmented, $p<0.05$, n=3 with 250 cells/each group). We next modulated NO levels by pretreating cells with either an NO donor (DPTA, 25 μM) or a NOS inhibitor (L-NAME, 100μM) to assess the relationship between NO and mitochondrial morphology. Neurons transfected with mt-dsRed (control) showed NO dependent mitochondrial morphological changes (vehicle 62.6±4, DPTA: 81.2±3, L-NAME: 46.3±2 elongated mitochondria). Neurons transfected with siRNA and WT-PHB showed similar mitochondrial morphology to controls (vehicle: 65.5±2, DPTA: 78.3±3, L-NAME: 54.5± 2), while neurons transfected with siRNA and CV-PHB did not respond to NO modulation (vehicle: 39.9±2, DPTA: 41.7±4, L-NAME: 39.7±1). These data indicated that a mutant CV-PHB fails to influence mitochondrial dynamics, suggesting that Cys^{69} is critical for the normal function of PHB in regulating mitochondrial fusion and fission in response to NO. Ongoing studies are addressing the mechanisms whereby Cys^{69} nitrosylation regulates PHB levels and function in neurons.

Disclosures: L. Qian: None. **Y. Qu:** None. **G. Manfredi:** None. **C. Iadecola:** None. **P. Zhou:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.04/Z6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: US Department of Veterans Affairs BLR&D IK2 BX001686

US Department of Veterans Affairs BX003083A

University of Minnesota Healthy Foods, Healthy Lives Institute

Minnesota Veterans Medical Research & Education Foundation

Title: Orexin A attenuates palmitic acid-induced hypothalamic cell death

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[1]Food Sci. and Nutr., Univ. of Minnesota, Saint Paul, MN; [2]Minneapolis Veterans Affairs Hlth. Care Syst., Minneapolis, MN; [3]Minnesota Obesity Ctr., Saint Paul, MN

Abstract: Palmitic acid (PA), an abundant dietary saturated fatty acid, contributes to obesity and hypothalamic dysregulation in part through increase in oxidative stress, insulin resistance, and neuroinflammation. Increased production of reactive oxygen species (ROS) as a result of PA exposure contributes to the onset of neuronal apoptosis. Additionally, high fat diets lead to changes in hypothalamic gene expression profiles including suppression of the anti-apoptotic protein B cell lymphoma 2 (Bcl-2) and upregulation of the pro-apoptotic protein B cell lymphoma 2 associated X protein (Bax). Orexin A (OXA), a hypothalamic peptide important in obesity resistance, also contributes to neuroprotection. Prior studies have demonstrated that OXA attenuates oxidative stress induced cell death. We hypothesized that OXA would be neuroprotective against PA induced cell death. To test this, we treated an immortalized hypothalamic cell line (designated mHypoA-1/2) with OXA and PA. We demonstrate that OXA attenuates PA-induced hypothalamic cell death via reduced caspase-3/7 apoptosis, stabilization of Bcl-2 gene expression, and reduced Bax/Bcl-2 gene expression ratio. We also found that OXA inhibits ROS production after PA exposure. Finally, we show that OXA treatment alters mHypoA-1/2 intracellular metabolism, resulting in increased basal respiration, maximum respiration, ATP production, and reserve capacity. Collectively, these results support that OXA protects against PA-induced hypothalamic dysregulation, and may represent one mechanism through which OXA can ameliorate effects of obesogenic diet on brain health.

Disclosures: C.M. Duffy: None. **J.P. Nixon:** None. **T.A. Butterick:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.05/Z7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF 2014-0420

NRF 2015R1A5A2008833

Title: A mitochondrial division inhibitor, Mdivi-1, inhibits mitochondrial fragmentation and attenuates kainic acid-induced hippocampal cell death

Authors: *H. KIM[1], J. LEE[1], K. PARK[2], W.-H. KIM[2], G. ROH[1];
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Abstract: Kainic acid (KA)-induced excitotoxicity promotes cytoplasmic calcium accumulation, oxidative stress, and apoptotic signaling, leading to hippocampal neuronal death. Mitochondria play a critical role in neuroinflammation and the oxidative stress response. Mitochondrial morphology is disrupted during KA-induced seizures; however, it is not clear whether mitochondrial fission or fusion factors are involved in KA-induced neuronal death. We investigated the effect of Mdivi-1, a chemical inhibitor of the mitochondrial fission protein Drp1, on mitochondrial morphology and function in KA-injected mice. Mdivi-1 pretreatment significantly reduced seizure activity and increased survival rates of KA-treated mice. Mdivi-1 was protective against mitochondrial morphological disruption, and it reduced levels of phosphorylated Drp1 (Ser616) and Parkin recruitment to mitochondria. By contrast, levels of mitochondrial fusion factors did not change. Mdivi-1 also reduced KA-induced neuroinflammation and glial activation. We conclude that inhibition of mitochondrial fission attenuates Parkin-mediated mitochondrial degradation and protects from KA-induced hippocampal neuronal cell death.

Disclosures: **H. Kim:** None. **J. Lee:** None. **K. Park:** None. **W. Kim:** None. **G. Roh:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.06/Z8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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

Keio University Grant-in-Aid for Encouragement of Young Medical Scientists

Title: Excessive D-serine mediates cell death in developing neuron

Authors: *M. SUZUKI, J. SASABE, S. AISO;
Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Neuronal cell death is observed in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. One possible mechanism of cell death is hyper-stimulation of glutamate receptors, which activates apoptotic pathway in neuron. D-serine is a co-agonist of *N*-Methyl-D-Aspartate type glutamate receptor (NMDAR), and modulates NMDAR activity. Although excessive D-serine is reported to cause hyper-activation of NMDAR and cell death, the mechanism is yet to be identified.
In this study, we found novel apoptotic stimulation on developing neurons. Treatment of D-serine in the absence of L-serine causes caspase-3 dependent apoptosis. D-serine mediated activation of caspase-3 in primary neurons at immature stage (DIV4) but not mature stage (DIV16). In addition, the cell death was occurred in cortical neurons but not in cerebellar neurons.
Although NMDAR excitotoxicity results in activation of neuronal nitric oxide synthase (nNOS), NOS inhibitor did not suppressed apoptosis. Inhibitor for NMDAR (MK-801 and 5,7-dichlorokynurenic acid), for AMPAR (DNQX), or Ca2+ chelator (EGTA) did not inhibit D-serine dependent cell death. However, only L-serine can completely suppressed D-serine dependent apoptosis.
Because L-serine is known as neurotrophic amino acids supplied from glial cells in central nervous system, D-serine might interfere with the neurotrophic effect of L-serine on neurons.

Disclosures: M. Suzuki: None. **J. Sasabe:** None. **S. Aiso:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.07/Z9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council of Canada Grant 355803-2013

Title: The adaptor protein p66shc regulates metabolism, ros production, and amyloid beta sensitivity in cns cells

Authors: *A. LONE[1], R. C. CUMMING[2];
[1]Biol., [2]Dept. of Biol., Western Univ., London, ON, Canada

Abstract: A key pathological feature of Alzheimer's disease (AD) is the accumulation of extracellular deposits of amyloid beta (Aß) peptide within the brains of affected individuals. Aß accumulation is associated with oxidative stress, extensive neuronal death and synaptic loss. However, 40% of the elderly have pronounced Aß deposition within their brains, yet show no symptoms of dementia, indicating that some cells are resistant to Aß toxicity. Several studies suggest that central nervous system (CNS) cells that are resistant to the harmful effects of Aß display a metabolic shift from mitochondrial-dependent oxidative phosphorylation (OXPHOS) to aerobic glycolysis for their energy needs. The adaptor protein p66SHC has been shown to play a definitive role in aging, as well as in regulation of redox balance and ROS levels. Recent studies have shown that p66SHC expression and activation can shift the cellular metabolic state from OXPHOS to aerobic glycolysis. Hence, we propose that the expression and activation of p66SHC in CNS cells promotes both increased OXPHOS and sensitivity to Aß toxicity. To test this hypothesis, we transiently overexpressed p66SHC in a rodent neuronal cell line, and knocked down endogenous p66SHC in a rodent glial cell line, to determine the effect of p66Shc activation on metabolic activity. Changes in mitochondrial ROS levels and mitochondrial electron transport chain (ETC) activity were also measured. We examined the subcellular localization of the phosphorylated and activated form of p66SHC. Lastly, we investigated if p66SHC expression and activation affected sensitivity to Aß in both neuronal and glial cell lines in an OXPHOS dependent manner. Transient overexpression of p66SHC repressed glycolytic enzyme expression and increased mitochondrial ETC activity and ROS levels. The opposite effect was observed when endogenous p66SHC expression was knocked down. Exposure to Aß promoted the phosphorylation and activation of p66SHC, resulting in an upregulation of mitochondrial metabolism. Moreover, expression and activation of p66SHC increased sensitivity to Aß toxicity. Our findings indicate that expression and activation of p66SHC renders CNS cells more sensitive to Aß toxicity by promoting mitochondrial OXPHOS while repressing aerobic glycolysis. Thus, p66Shc may represent a therapeutically relevant target for the treatment of AD.

Disclosures: **A. Lone:** None. **R.C. Cumming:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.08/Z10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grant-in-Aid for Scientific Research (KAKENHI)

Title: Genetic manipulation of mTORC1 signaling in mouse cerebellar Purkinje cell

Authors: ***H. KASSAI**[1], Y. SAKAI[1], H. NAKAYAMA[4], T. MAEDA[2], K. HASHIMOTO[4], M. KANO[3], A. AIBA[1];
[1]Grad. Sch. of Med., [2]Inst. of Mol. & Cel. Biosci, [3]Univ. of Tokyo, Tokyo, Japan; [4]Hiroshima Univ., Hiroshima, Japan

Abstract: Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase, and functions as two distinct complexes, termed mTORC1 and mTORC2. The mTORC1 pathway is activated by amino acids or growth factor stimuli, and plays a central role in cell growth processes such as protein synthesis and autophagy. Abnormal mTOR signaling is often found in many neurological diseases such as tuberous sclerosis, autism and neurodegenerative diseases. Since all these diseases are associated with chronic activation of mTORC1 pathway, we focused on addressing mTOR functions in these diseases by establishing animal models of chronically activated mTORC1 pathway in cerebellar Purkinje cells. We used constitutively active mutant of mTOR, which retains its kinase activity even under starvation conditions. We crossed two lines of transgenic (Tg) mice; TRE-mTOR Tg in which active mTOR is linked to TRE promoter, and L7-tTA Tg in which tTA expression is driven by Purkinje-cell specific L7 promoter. In double Tg mice (L7-mTOR Tg mice, hereafter), expression of active mTOR in Purkinje cells did not affect the gross appearance or fertility. However, we found many abnormalities in the morphology of Purkinje cells in L7-mTOR Tg mice, such as enlarged soma, thickened dendrites and increased number of self-crossings. In addition, the number of Purkinje cells began to decrease by apoptotic cell death from 3 weeks of age in the Tg mice. These results indicate that mTORC1 signaling is important for morphology and survival of cerebellar Purkinje cells.

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Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.09/Z11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Bcl-XL knockout attenuates mitochondrial respiration and shifts cellular metabolism towards the pentose phosphate pathway

Authors: *A. METHNER[1], A. PFEIFFER[2], J. SCHNEIDER[2], A. DOLGA[3], T.-D. VOSS[4], J. LEWERENZ[4];
[1]Johannes Gutenberg Univ. Mainz, Mainz, Germany; [2]Johannes Gutenberg Univ., Mainz, Germany; [3]Univ. of Groningen, Groningen, Netherlands; [4]Univ. of Ulm, Ulm, Germany

Abstract: Bcl-XL is an anti-apoptotic protein mainly localized to the outer mitochondrial membrane which enhances mitochondrial bioenergetics by controlling Ca^{2+} influx into the mitochondria. Here, we performed an in-depth analysis of the effect of mitochondrial Bcl-xL on mitochondrial shape and function in well-characterized knockout (KO) and rescue fibroblast cell lines. KO mitochondria were more fragmented and exhibited reduced oxidative phosphorylation and electron transfer capacity with convergent complex I and II electron input suggesting a predominance of other means of ATP generation like glycolysis or the pentose phosphate pathway (PPP). Inhibition of ATP synthase with oligomycin treatment indeed resulted in an instant increase in acidification of the growth medium in line with an instant switch to glycolysis. In the presence of galactose, KO cells were also more susceptible to mitochondrial toxins. Under steady-state conditions, however, KO cells did not have an increased glycolytic activity. Apparently, these cells can turn on glycolysis more efficiently than wildtype cells on demand. The pentose phosphate shunt is another major pathway used by cells to generate NADPH that can be used to reduce oxidized glutathione (GSH), a major cellular antioxidant. It also has a prominent anabolic function by generating phosphopentoses and ribonucleotides. KO cells had an increased abundance and function of the rate-limiting enzyme glucose-6-phosphate dehydrogenase, more NADPH, more GSH and an increased growth rate but were not protected against oxidative stress. We therefore concluded that reduced mitochondrial respiration caused by malfunctioning mitochondria lacking Bcl-XL is compensated by increased use of glucose in the pentose phosphate shunt.

Disclosures: **A. Methner:** None. **A. Pfeiffer:** None. **J. Schneider:** None. **A. Dolga:** None. **T. Voss:** None. **J. Lewerenz:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

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Program#/Poster#: 419.10/Z12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council of Canada Grant 355803-2013

Title: Investigating the protective effects of mitochondrially targeted telomerase on neuronal metabolism and resistance to amyloid-beta

Authors: *O. S. SINGH, R. C. CUMMING;
Dept. of Biol., Western Univ., London, ON, Canada

Abstract: Telomerase consists of two main components that function as dimers; the telomerase RNA component (TERC) and the telomerase reverse transcriptase (TERT). TERT catalytically adds TTAGGG hexanucleotide repeats to the 3' end of the lagging strand of chromosomes thereby preventing telomere shortening during DNA replication in mitotic cells. While telomerase is highly expressed in germ line cells, embryonic stem cells, and malignant cancer cells, there is increasing evidence of a non-telomeric function of TERT in post-mitotic cells. Recent studies have revealed that extra-nuclear TERT may protect neurons from apoptosis, ischemic cell death, N-methyl-D-aspartate receptor (NMDA)-induced neurotoxicity, and glutamate cytotoxicity. More importantly, when cells experience oxidative stress, TERT can translocate from the nucleus to the mitochondria and promote a decrease in mitochondrial reactive oxygen species (ROS) production while increasing mitochondrial membrane potential. Mitochondrial localized TERT has been detected in both primary neuronal cultures and in hippocampal neurons of Alzheimer's disease (AD) brains. Mitochondrial dysfunction is a prominent feature of many neurodegenerative diseases including AD. A major pathological feature of AD is the progressive accumulation of amyloid-beta (Aβ) peptide within the cortex and hippocampus. It has been shown that Aβ oligomers directly interfere with mitochondrial respiration by binding to alcohol dehydrogenase (ABAD) thereby promoting mitochondrial dysfunction, ROS production, and cell death. Hence, we hypothesized that TERT may protect neurons from mitochondrial dysfunction and Aβ toxicity via metabolic reprogramming. We have created stable cell cultures with doxycycline inducible expression of TERT in the murine hippocampal neuronal cell line HT-22. Induction of TERT expression in HT22 cells resulted in altered glycolytic enzyme activity, mitochondrial membrane potential and ROS production. We are currently determining if TERT localizes to mitochondria and confers neuroprotection against Aβ toxicity via metabolic reprogramming. The results from this study may lead to a novel potential therapy for the treatment of AD.

Disclosures: O.S. Singh: None. **R.C. Cumming:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.11/Z13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP

CNPQ

CAPES

Title: Role of motochondrial oxidative stress and UCP2 in epilepsy experimental model induced by pilocarpine

Authors: *M. B. NEJM[1], M. MARQUES, 04034032[2], A. HAIDAR[2], F. SCORZA[2], E. CAVALHEIRO[2];
[1]Federal Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Federal Univ. of Sao Paulo, São Paulo, Brazil

Abstract: Temporal lobe epilepsy (TLE) is the most common form of epilepsy in humans. Evidence supports the fact that neuronal injury observed in epilepsy may be the result of an overproduction of free radicals (oxidative stress) and related to excitotoxicity event. Mitochondria is responsible for ATP synthesis but also is involved in calcium uptake and release, transient mitochondrial permeability and in ROS (Reactive Oxygen Species) generation. It is known that excessive ROS generation can promote ultrastructural changes in the respiratory chain. The first uncoupling protein mitochondrial of respiratory chain (UCP - Uncoupling Mitochondrial Protein) described was UCP1 and others subsequently were discovered. UCP2 is significantly expressed in certain brain regions and is responsible for stabilizing the mitochondrial membrane potential, an important event suggested to reduce cell death. It was suggested that activation of UCP2 may protect neurons in degenerative processes associated with oxidative stress and mitochondrial changes in potential trasmembrana so, may be therefore, neuroprotective or the brain. Antioxidant agents, at low concentrations compared with the oxidizable substrate, can reduce or prevent oxidation, also helping to combat oxidative stress. This raises the proposal to clarify from the beginning of epileptogenesis and over the periods of pilocarpine experimental model of epilepsy, UCP2 participation using small interfering RNA for this protein. We aim to evaluate: phenotypic changes on pilocarpine experimental model (mortality, latency to develop Status epilepticus (SE) and frequency of seizures); expression of UCP2 and antioxidant enzymes (MnSOD and GPx) in hippocampus mitochondria; enzymatic activity of respiratory chain complexes; presence of neuronal death; production of ROS; and morphology of these mitochondria. So far, the obtained results show efficacy in UCP2 silencing verified by the reduction of protein expression. We can therefore speculate about UCP2

neuroprotective role since these animals showed high mortality and reduced latency to develop the SE; also it were showed increased neuronal death and ROS production, including from mitochondrial origin. The silencing, even occasionally, promoted important changes. With the results that we will still get we intend to elucidate the neuroprotective role of UCP2 as long-term silencing effects in animals from the chronic group.

Disclosures: **M.B. Nejm:** None. **M. Marques:** None. **A. Haidar:** None. **F. Scorza:** None. **E. Cavalheiro:** None.

Poster

419. Apoptosis and Mitochondria

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS35533

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NIH Grant NS055088

BrightFocus Foundation

Title: Epigenetic regulation of endophilin-B1 promotes neuronal viability in response to disease and injury

Authors: ***R. S. MORRISON**[1], D. B. WANG[1], Y. KINOSHITA[1], C. KINOSHITA[1], R. LEE[1], S. P. MURPHY[1], B. L. SOPHER[2], G. A. GARDEN[2];
[1]Dept Neurolog. Surgery, [2]Dept of Neurol., Univ. Washington, Seattle, WA

Abstract: Epigenetic changes in gene expression are implicated in neuronal dysfunction associated with neurodegenerative and acute brain disorders as well as normal aging. Histone deacetylase-2 (HDAC2) is a member of an enzyme family that catalyzes the removal of acetyl groups on lysine residues of histone proteins, leading to altered chromatin structure and gene expression. HDAC2 is upregulated during neuronal differentiation, comprising the major HDAC protein in adult brain. In Alzheimer's disease (AD), HDAC2 is elevated, repressing expression of several genes necessary for synaptic function, learning and memory. However, the impact of elevated HDAC2 on neuronal gene expression in AD, aging brain or neuronal injury remains poorly understood. Here we demonstrate that HDAC2 overexpression in cultured primary cortical neurons represses expression of endophilin B1, also known as Bax-interacting factor-1

(Bif-1). We recently showed that Bif-1 is a neuroprotective protein that stimulates mitochondrial elongation. Importantly, we demonstrated that neuron-specific isoforms of Bif-1 are reduced in human AD cortex and CNS tissue from mouse models of AD and ischemic stroke. HDAC2 overexpression also enhanced caspase 3 activation and repressed expression of mitofusin 2, a protein that promotes mitochondrial fusion. We further demonstrate that HDAC2 knockdown in mouse primary cortical neuron cultures protects against beta-amyloid induced mitochondrial damage, caspase activation and apoptosis while restoring Bif-1 protein levels. The protective effect of HDAC2 knockdown was abrogated by Bif-1 shRNA and in Bif-1 deficient neurons. HDAC2 genetic deficiency also enhanced neuronal survival and minimized the loss of Bif-1 in the middle cerebral artery occlusion model of cerebral ischemia. HDAC2-dependent regulation of Bif-1 was neuron-specific and not observed in mouse embryo fibroblasts. These findings support the hypothesis that HDAC2 represses Bif-1, a critical neuroprotective protein, and other regulators of mitochondrial dynamics, sensitizing neurons to stress-induced mitochondrial dysfunction and apoptotic cell death.

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Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.13/AA1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Altered molecular processes of malignant peripheral nerve sheath tumors support the PTP closure state driving anti-apoptotic tumor progression

Authors: *D. DANIELS[1], B. C. PRUDNER[2], B. VAN TINE[2];
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Abstract: Sarcomas are malignant tumors of mesenchymal origin that arise from lymphatic, circulatory and connective tissue. Of the 13,000 soft tissue and bones cases diagnosed in the US, approximately 1% develop malignant tumors annually (McFarland 2015). Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are atypical soft tissue sarcomas of neural origin that engender high morbidity in the afflicted when resection is not achievable. 50% of MPNSTs are NF1 associated and the other half arise spontaneously, creating a lifetime risk that 10% of NF1 patients will develop MPNSTs (Ratner 2015). Current therapeutics such as chemotherapy and radiation have minimal impact in MPNSTs and readily kill normal cells, therefore, cutting edge

sarcoma research has migrated to highly specific, antagonistic molecular modeled therapeutics.In cells, the mitochondria is known to function as the key energy generating and autophagic organelle and in cancer cells these entities have an even more prominent role in cellular function, metabolism and cell death (Verschoor 2012). Under stressful conditions normal cells undergo autophagic processes such as apoptosis to prevent the advancement of mutant cell survival and viability via mitochondrial membrane manipulation. Cancer cells have the unique capability to evade these auto-regulatory mechanisms thus promoting tumorigenesis. In normal cells the apoptotic mechanism, mitochondrial permeability transition pore (PTP) opening is directed by a series of molecular signals: mitochondrial depolarization, respiratory inhibition and generation of reactive oxygen species (ROS), release of Ca2+, swelling of the mitochondria leading to breaches in the outer membrane that engender the release of intermembrane protein, that brings about cell death (Rasola 2014). The induction of systems that stimulate PTP opening characteristics serve as promising models for the promotion of cell death in malignant tumors that have adopted anti-apoptotic traits such as MPNSTs.

Disclosures: **D. Daniels:** None. **B.C. Prudner:** None. **B. Van Tine:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.14/AA2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ting Tsung & Wei-Fong Chao Foundation

John S Dunn Research Foundation

Title: Integrating systems biology and experimental neurology analysis to discover ovary-orientated protein ociad1 in alzheimer pathogenesis

Authors: *X. LI[1,2], T. LIU[1], L. WANG[1], Z. YIN[1,2], M. D. CYTOKOWISC[3], A. L. RIVERA[3], J. J. MANCUSO[1,2], H. ZHAO[1], S. POWELL[3], W. XIA[2,5], S. T. C. WONG[1,2,3,4];
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Abstract: A better understanding of the mechanisms by which early pathological events (e.g., amyloid deposit) cause neurodegeneration in Alzheimer disease (AD) can help unravel the

pathogenesis of this complex heterogeneous disorder, and consequentially develop new therapeutic strategies. Integrating systems biology and experimental neurology approaches, we discovered the potential of an ovary-orientated protein OCIAD1 (Ovarian-Carcinoma-Immunoreactive-Antigen-Domain-Containing-1) as an etiopathological contributor in AD. The association between OCIAD1 and neurodegeneration in AD is revealed by analyzing the brain vulnerability-relevant gene signatures in sporadic AD patients and the functional partner network *in silico.* This is consistent to our previous finding in synaptic proteomics during disease progression in AD transgenic mice. In humans, OCIAD1 is co-expressed with several AD-associated genes in the brain, at particularly higher levels in the vulnerable brain areas correlated with disease severity. Under conditions mimicking early AD pathological changes, OCIAD1 is elevated in the neurons and partly associated with Aβ/GSK-3β/CTNNB1 signaling pathway. Moreover, an interaction between OCIAD1 and BCL-2 impairs the membrane potential of mitochondria, facilitating release of mitochondrial apoptotic factors and caspase 3 activation. Notably, knocking down OCIAD1 mitigates the aggravating effect of Aβ on apoptosis after exposure of multiple stressors. Our findings indicate that OCIAD1 is a common etiological factor linking brain vulnerability in AD to mitochondria dysfunction.

Disclosures: **X. Li:** None. **T. Liu:** None. **L. Wang:** None. **Z. Yin:** None. **M.D. Cytokowisc:** None. **A.L. Rivera:** None. **J.J. Mancuso:** None. **H. Zhao:** None. **S. Powell:** None. **W. Xia:** None. **S.T.C. Wong:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.15/AA3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 104-2320-B-570-002

Title: The highly electronegative low-density lipoprotein l5 impairs the neuritogenesis in ngf-induced pc12 cells

Authors: ***C.-T. LEE**[1], C.-L. LAI[2], J.-Y. WANG[2];
[1]Hsin Sheng Junior Col. of Med. Care and Mana, Taoyuan City, Taiwan; [2]Grad. Inst. of Medicine, Col. of Medicine,Kaohsiung Med. Univ., Kaohsiung, Taiwan

Abstract: The patients with cardiovascular diseases (CVD) are often associated with the occurrence of cognitive decline and dementia. Low-density lipoprotein (LDL), which is one of the risk factor for CVD, may play a critical role; further, L5, a electronegative LDL with

relatively highest electronegative charge, is the most important candidate. So far, the biological effect of electronegative LDL on neuronal cell reactivity is fully unclear and has not been studied. Here, an in vitro cultured model of PC12 cell, a rat pheochromocytoma-derived cell line with neuronal characteristics, was used in this study. We treated the cells with L5, L1 (an LDL with lesser extent of electronegativity than L5) and oxidized LDL (an artificially synthesized LDL), and assayed for cytotoxicity and neuritogenesis. The results indicated that both oxLDL and L5 are toxic to PC12 cells in a dose-dependent manner. L1 exerted no effect on cell viability, hence exhibiting a non-neurotoxic characteristic. The toxicity of L5 at 50 μg/ml was not appeared until the cultures were treated for more than the duration of 24 h. Moreover, both L5 and oxLDL at 30 μg/ml, but not L1, leaded to the failure of NGF-induced neuritogenesis in PC12 cells, detected by neurite outgrowth assay. Further, we identified that L5 does not affect NGF receptor activity, but downregulates PI3K/Akt signaling, consequently impairing NGF-induced neuritogenesis. These results suggested that L5 itself has the cytotoxicity and impairs the neuritogenesis in NGF-induced neuron-like PC12 cells.

Disclosures: **C. Lee:** None. **C. Lai:** None. **J. Wang:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 419.16/AA4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Manitoba

Winnipeg Health Sciences Center

Title: PARP-1-dependent expression of pro-apoptotic factor bnip3 is mediated by hypoxia inducible factor-1α in hypoxic neurons

Authors: P. LU, S. ATOUI, *C. M. ANDERSON;
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Abstract: Oxidative genotoxicity in brain ischemia/hypoxia causes excessive activity of the nuclear enzyme poly(ADP-ribose) polymerase-1. The catalytic activity of PARP-1, in turn, leads to depletion of NAD^+, accumulation of ADP-ribose polymers, mitochondrial permeability and nuclear translocation of apoptosis-inducing factor (AIF). We recently demonstrated that the pro-death Bcl-2 protein family member Bcl-2/adenovirus E1B 19 kDa-interacting protein (Bnip3) acts as a mitochondrial mediator of PARP-1-induced mitotoxicity and neuron death. Here we

hypothesized that PARP-1-induced NAD^+ depletion inhibits sirtuin deacetylase activity, leading to enhanced acetylation-mediated HIF-1α stability and HIF-1-mediated transcription of Bnip3. Activation of PARP-1 by exposure of primary cortical neurons to hypoxia or normoxic genotoxic concentrations of the DNA alkylating agent, 1-methyl-3-nitro-1-nitroguanidine (MNNG), caused time-dependent depletion of cytoplasmic/nuclear NAD^+ levels, inhibition of sirtuin deacetylase activity and hyper-acetylation of HIF-1α. Chromatin immunoprecipitation using an anti-HIF-1α antibody, followed by qPCR, also revealed significant enhancement of HIF-1α binding to a HIF-responsive element in the Bnip3 upstream promoter region. All of these events, along with increased Bnip3 transcript levels, were mitigated in the presence of the PARP-1 inhibitor, PJ34 (10 μM), or in *parp-1*$^{-/-}$ neurons. This confirms PARP-1 dependence of hypoxic Bnip3 regulation and verifies a direct linkage between PARP-1 and Bnip3 regulation by HIF-1 following MNNG treatment. Moreover, silencing of HIF-1α using lentiviral shRNA delivery significantly reduced hypoxic HIF-1/Bnip3 HRE interaction and Bnip3 transcript levels, thereby demonstrating a direct role of HIF-1α in hypoxic Bnip3 expression. Together, these data suggest a regulatory role for HIF-1 in neurotoxic Bnip3 expression following brain injury resulting in hypoxia/ischemia.

Disclosures: **P. Lu:** None. **S. Atoui:** None. **C.M. Anderson:** None.

Poster

419. Apoptosis and Mitochondria

Location: Halls B-H

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Program#/Poster#: 419.17/AA5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant

EY012245

Title: Rescue of visual function resulting from mitochondrial defects

Authors: *G. CORTOPASSI[1], S. DATTA[2], A. YU[2];
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Abstract: Inherited mitochondrial defects cause neurodegeneration of neurons with long axons. Leber's hereditary Optic Neuropathy (LHON) is an inherited mitochondrial orphan disease causing complex 1 deficiency and vision loss resulting from Retinal Ganglion Cell death. We characterized vision loss in an animal model of mitochondrial disease, the Ndufs4KO mouse with a severe defect in mitochondrial complex 1. Retinal ganglion cell defects were determined

by MultiElectrodeArray (MEA) recording, and Electroretinogram (ERG) recording, and had obvious defects from day 30 on. RNAseq analysis of retinas demonstrated an 'inflammatory wave' that was coincident with vision loss, and preceded Retinal Ganglion Cell loss. We took a repurposing strategy to identify potential LHON therapeutics. We identified a specific biochemical defect in LHON cybrids, i.e. deficiency in Complex 1-dependent ATP synthesis, and screened 1600 FDA/EMA approved drugs for protection. This screening identified ultimately 2 drugs that dose-dependently protect from the LHON-dependent complex 1 ATP synthetic deficiency. These 2 drugs appear to hit the same mitochondrial target, in that agonists of that target protect and antagonists block the ATP synthetic rescue. The two drugs were tested for benefit in the Ndufs4 model of LHON. The two drugs provided significant protection from vision loss as measured by visual cliff and Electroretinograms, and rescue from the retinal 'inflammatory wave' that coincides with complex 1-dependent functional visual loss and retinal ganglion cell death. These results suggest that a repurposing strategy can identify novel leads with potential benefit for LHON therapy. Yu et al., (2015) Mitochondrial complex I deficiency leads to inflammation and retinal ganglion cell death in the Ndufs4 mouse.

Disclosures: **G. Cortopassi:** None. **S. Datta:** None. **A. Yu:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.01/AA6

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01NS50465

Title: Enhancing brain metabolism to restore functional connectivity and plasticity after TBI

Authors: *G. KRISHNA[1], Z. YING[1], L. F. F. ROYES[3,1], A. PAYDAR[2], N. G. HARRIS[2], F. GOMEZ-PINILLA[1,2];
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Abstract: The post-TBI metabolic depression impairs the ability of neuronal circuits to comply with activity demands, limiting the success of rehabilitative strategies, particularly during the acute period of TBI, which is critical for athletes to return to play, or patients to engage in rehabilitation programs. Although most neurons survive mild or moderate TBI, they cannot operate efficiently, and this severely compromises brain function. Our paradigm is based on the

concept that TBI disrupts brain functional connectivity (fc), and that synaptic function and cell metabolism are underlying substrates of fc. We capitalize on the enhancing action of flavonoid derivative, 7,8-dihydroxyflavone (7,8-DHF, BDNF analog) on cell metabolism and synaptic plasticity to restore fc. We performed moderate fluid percussion injury (FPI) and 7,8-DHF (5 mg/kg, ip) was administered in animals receiving FPI with or without exposure to voluntary exercise. TBI resulted in disturbances in energy homeostasis markers (PGC-1α, COX II) and plasticity/growth (GAP-43, synaptophysin), concurrent with reductions in memory in Barnes maze. Treatment with 7,8-DHF or exercise ameliorated impairments in cognitive function and energy homeostasis, and enhanced the activation of TrkB BDNF receptor signaling. The combined action of exercise and 7,8-DHF resulted in elevated values in most of the variables (in an additive fashion). Resting state functional MRI (rsfMRI) data acquired from the same groups to monitor circuit-level changes associated with functional brain reorganization showed that the action of 7,8-DHF improved brain circuit connectivity (nodal connection strength) compared to FPI alone, where it is was reduced compared to sham animals. Exercise and 7,8-DHF also normalized connectivity suggesting their potential to promote compensatory recovery of existing circuits. These data show that 7,8-DHF can render as a prototype molecule that links metabolism and synaptic plasticity, with the potential to normalize functional brain connectivity crucial to enhance the effects of training on TBI pathology. These findings further elucidate the functional implications of exercise and 7,8-DHF to provide novel perspective into neurorehabilitative strategy.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.02/AA7

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: 09SDG2060701

NS37853

NS36645

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Title: Perivascular macrophages mediate vascular oxidative stress and neurovascular dysfunction induced by amyloid-β through CD36 and NOX2

Authors: *L. PARK[1], L. GARCIA-BONILLA[1], K. UEKAWA[1], P. ZHOU[1], R. PITSTICK[2], L. YOUNKIN[3], S. YOUNKIN[3], G. CARLSON[2], C. IADECOLA[1];
[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]McLaughlin Res. Inst., Great Falls, MT; [3]Mayo Clin. Jacksonville, Jacksonville, FL

Abstract: Aβ has harmful effects on the cerebral microcirculation, which may contribute to cognitive impairment in Alzheimer disease and mixed dementias. The cerebrovascular effects of Aβ are mediated by reactive oxygen species (ROS) from a NOX2-containing NADPH oxidase activated by the interaction of Aβ with the innate immunity receptor CD36 (PNAS, 110:3089-3094, 2013). However, the cellular source(s) of the ROS have not been established. We tested the hypothesis that perivascular macrophages (PVM), myeloid cells located within the perivascular spaces and expressing CD36 and NOX2, contribute to the oxidative stress and cerebrovascular dysfunction induced by Aβ. Cerebral blood flow (CBF) was measured by laser-Doppler flowmetry in the somatosensory cortex of urethane-chloralose anesthetized male mice (age 3-4 months; n=5/group). PVM were depleted by icv injection of liposomes containing clodronate or PBS (vehicle), and tested its effect 7 days later. In mice injected with PBS liposomes, neocortical superfusion with $A\beta_{1-40}$ (5 μM) elevated ROS by 78±11% (Mean±SE; $p<0.05$) and attenuated the CBF increase produced by whisker stimulation (WS; -41±3%) or neocortical application of the endothelium-dependent vasodilator acetylcholine (ACh; -36±4%)($p<0.05$). PVM depletion (-60±4%) prevented oxidative stress and the attenuation in cerebrovascular responses both in WT mice treated with Aβ and in tg2576 mice ($p>0.05$ from controls). Since PVM are bone marrow (BM) derived, we used BM chimeras to delete CD36 or NOX2 in PVM. In WT mice transplanted with WT BM (wt-->◊wt), Aβ superfusion increased ROS in PVM by 80±8% ($p<0.05$) and attenuated CBF responses (WS: -32±2%; ACh: -24±3%; $p<0.05$ from vehicle). However, in WT mice receiving $CD36^{-/-}$ or $Nox2^{-/-}$ BM Aβ failed to increase ROS and to impair the CBF responses ($p>0.05$ from vehicle). Similarly, CBF responses were ameliorated in tg2576 mice receiving $CD36^{-/-}$ ($CD36^{-/-}$-->tg) or $Nox2^{-/-}$ BM ($Nox2^{-/-}$-->tg) ($p>0.05$ from wt-->◊wt), an effect associated with reduced vascular oxidative stress. Brain $A\beta_{1-40}$ levels did not differ between wt-->◊tg (4316±240 pmol/g) and $CD36^{-/-}$-->◊tg (3862±300 pmol/g) or $Nox2^{-/-}$-->◊tg (3781±313 pmol/g; $p>0.05$). We conclude that PVM are the critical cells required for the ROS production underlying the cerebrovascular dysfunction induced by Aβ, an effect mediated by CD36 and NOX2. These observations provide evidence that NOX2 and CD36 in PVM may be therapeutic targets to counteract the detrimental neurovascular effects of Aβ.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.03/AA8

Topic: C.09. Brain Injury and Trauma

Title: Calpain 5, an overlooked calpain in CNS injuries

Authors: *J. W. GEDDES, V. BONDADA, C. MASHBURN, J. A. WANG, R. L. HILL, E. D. HALL, D. W. RODGERS;
Spinal Cord & Brain Inj Res. Ctr., Univ. Kentucky Med. Ctr., Lexington, KY

Abstract: Calpains are Ca^{2+}-activated, neutral proteases implicated in neurodegeneration following acute insults such as stroke and traumatic brain injury, as well as in neurodegenerative disorders such as Alzheimer's disease. Calpain research in the CNS has focused almost exclusively on the classical calpains, Calpains 1 and 2. Calpain 5 (CAPN5), which lacks the penta-EF hand domain of classical calpains, is highly expressed in the CNS. Calpain 5 has homology to other calpains in the cysteine protease domain and also has a unique C2 domain at the C-terminus. Many, but not all, C2 domains act as Ca^{2+}-sensitive enzyme activators, and are also involved in binding to membranes and protein-protein interactions. The only other mammalian calpain with a putative C2 domain is CAPN6, which is non-catalytic. CAPN5 is difficult to detect in the cytosol, but is found in nuclear and crude mitochondrial fractions. It is Ca^{2+} activated, as demonstrated by loss of activity following incubation with EGTA and mutation of the Cys residue at the active site. CAPN5 is activated for up to 72h following traumatic brain injury in rats (controlled cortical impact, 2.2 mm depth, 3.5 m/sec), resulting in enzyme autolysis. To determine if the putative CAPN5 C2 domain binds Ca^{2+} and participates in enzyme activation, we first identified possible Ca^{2+} binding sites by structural modeling then mutated the Glu/Asp residues and examined activity of recombinant human CAPN5 in stable cell lines. Modeling revealed that the C2 domain of CAPN5 is similar to that of extended synaptotagmin 2 and contains two putative Ca^{2+} binding sites, Asp431 and Asp589, as well as a Glu residue (590) which may facilitate Ca^{2+} binding. A single mutation, D589N, markedly reduced CAPN5 activity and also altered CAPN5 localization. Together, these results demonstrate that the CAPN5 C2 domain binds Ca^{2+}, contributes to enzyme activation, and influences localization. Based on its high CNS expression, and activation following acute insults such as TBI, CAPN5 must be considered along with classical calpains 1 and 2 when evaluating the roles of calpains following neurotrauma and other CNS disorders.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Program#/Poster#: 420.04/AA9

Topic: C.09. Brain Injury and Trauma

Support: LLU School Of Medicine Seed Grant Funds

NIH R25 GM060507

DOD DCMRP DR080470

Title: Omega-3 fatty acids influence the onset and course of PTSD-like brain and behaviors following mild traumatic brain injury

Authors: A. OBENAUS[1], I. ALICEA-POLANCO[2], E. HADDAD[1], P. KALYAN-MASIH[2], J. D. VEGA-TORRES[2], E. KINNEY-LANG[1], M. DE LEON[2], *J. D. FIGUEROA[2];
[1]Pediatrics, [2]Ctr. for Hlth. Disparities and Mol. Med., Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: There is now substantial evidence that mild traumatic brain injury (mTBI) increases the risk of developing post-traumatic stress disorder (PTSD). Although the mechanisms by which risk of PTSD may be increased following mTBI remain unclear, it has been hypothesized that disruption of hippocampal homeostasis following injury may exacerbate PTSD symptoms. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are potent regulators of hippocampal function and have emerged as promising therapeutic agents for mTBI. The present study: (1) examines the efficacy of n-3 PUFAs to ameliorate PTSD-like behaviors at 4 and 8 weeks following mild controlled cortical impact (CCI) TBI in adult rats; (2) evaluates the impact of dietary n-3 PUFAs on hippocampal structure following chronic mTBI; (3) investigates the impact of omega-3 supplementation on hippocampal glucocorticoid signaling. Rats were fed with either control chow or chow enriched with n-3 PUFAs (750 mg/kg/day) for 4 weeks before being subjected to a single mTBI. We used a controlled cortical impact (CCI; 4 mm diameter tip, 0.5 mm depth, 6.0 m/s speed, 200 ms dwell) to induce mTBI. Animals were allowed to survive for 8 weeks after trauma and the brains collected for high-resolution magnetic resonance imaging (MRI). We found that consumption of n-3 PUFA significantly ameliorated functional deficits in locomotion (CatWalk XT gait analyses), sensorimotor gating (pre-pulse inhibition; PPI), nociception (mechanical-conflict avoidance), and anxiety-like behaviors (open field and elevated plus maze) following mTBI. Magnetic resonance imaging (MRI) demonstrated that dietary n-3 PUFAs preserved hippocampal volume when compared to animals fed with the control chow at 8 weeks post-injury ($p < 0.05$). Western blot and immunofluorescence analyses revealed a significant interplay between n-3 PUFA consumption and the FK-506-binding protein (FKBP51) levels in

the hippocampus. Collectively, our study demonstrates that the pathophysiological responses associated with PTSD are ameliorated by n-3 PUFAs consumption following mTBI.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Program#/Poster#: 420.05/AA10

Topic: C.08.Stroke

Support: NCSF:81400956

Shanghai Rising Star Program:16QA1402600

Title: Interleukin-2 monoclonal antibody(JES6-1) attenuates cerebral ischemic injury via modulating T cell activation

Authors: ***Y. ZHOU**[1], P. LI[2], L. WANG[2], W. YU[2];
[1]anesthesiology department, Renji Hosp., shanghai, China; [2]Renji Hospital, Shanghai Jiaotong Univ. Sch. of Med., Shanghai, China

Abstract: Background: Interleukin -2 can modulate effector T cells and regulatory T cells through different protein binding sites to have different effects. T lymphocyte infiltration within the central nervous system mediated immune inflammatory response to neuronal survival and long-term neurological function after cerebral ischemia. We therefore investigated the effect of Interleukin-2 mAb (JES6-1) attenuating post-ischemia inflammation and improving the long-term neurological function. Methods and Results: We used animal models for experimental brain ischemia as a paradigm of acute brain lesions and inject IL-2 mAb (JES6-1)2 hours after ischemia. We demonstrated that IL-2 mAb (JES6-1) administration decrease the lesion size 3 days after ischemia and improve neurological functions lasting out to 4 weeks. The neuroprotection also includes decreased infiltration of peripheral inflammatory cells into lesioned brain. IL-2 mAb (JES6-1) can upregulate the Treg cell amount in the early stage of ischemia. Conclusion: IL-2 mAb (JES6-1) regulates the activation status of T cells and protect against ischemia. It may serve as a potent target for further stroke treatment after acute phase by regulate the activation of T cells.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.06/AA11

Topic: C.09. Brain Injury and Trauma

Support: NSERC

CHN Foundation

Title: Acute upregulation of bone morphogenetic protein 4 regulates endogenous cell response and glial reactivity following spinal cord injury

Authors: *C. HART[1], S. M. DYCK[1], K. T. SANTHOSH[1], D. MILLER[2], S. KARIMI-ABDOLREZAEE[1];
[1]Physiol. and Pathophysiology, [2]Pharmacol. and Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Activation of astrocytes is a hallmark of spinal cord injury (SCI) that plays a key role in endogenous repair processes after injury. Reactive astrocytes modulate the injury microenvironment by producing a plethora of inhibitory factors. In this study, we demonstrate that activated astrocytes influence the properties of neural stem/progenitor cells (NPCs) by upregulating bone morphogenetic protein 4 (BMP4). BMP4 is a well known morphogen in the developing CNS with an established role in modulating astrocyte differentiation. In a clinically-relevant model of compressive SCI in rats, we found that BMP4 is robustly upregulated in the acute phase of injury between 1 and 3 days post injury. The temporal upregulation of BMP4 is correlated with several key events in acute SCI including vascular disruption and blood-spinal barrier permeability as well as activation of endogenous precursor cells. To date, the role of BMP4 in acute SCI has remained largely unknown. NPCs, glia and endothelial cells were all shown to express BMP receptors, suggesting they are responsive to changes in BMP4 expression. Here, using complementary *in vitro* and *in vivo* approaches, we sought to unravel the ramification of BMP4 upregulation on the repair process in acute rat SCI. Our findings suggest that reactive astrocytes are a significant source of BMP4 *in vitro* in response to IL-1β+TNFα activation, but not to LPS or TGFB treatment. Interestingly, microglia decreased their BMP4 expression upon activation. NPCs treated with BMP4 significantly decreased their proliferation and predominantly differentiated into astrocytes. These effects were inhibited when BMP4 was co-treated with noggin, a natural neutralizer of BMP4, in a concentration-dependent manner. In cultures of reactive astrocytes, BMP4 itself did not seem to be a global activator of astrocytes as there was no effect on their pro-inflammatory cytokine or nitric oxide production. However, BMP4 remarkably increased the production of inhibitory chondroitin sulfate proteoglycans (CSPGs). We also found that endothelial cells increase their expression of BMPER (BMP

binding endothelial regulator) in the acute phase of SCI suggesting a link between astrocyte and endothelial activity after injury. In conclusion, our findings indicate that SCI-induced upregulation of BMP4 impacts several cell populations in the injured spinal cord, by inducing astrocyte differentiation of NPCs, CSPG production by reactive astrocytes and may have a role in modulating vascular endothelial cells through its interaction with BMPER. Accordingly, our findings identify a role for BMP4 in modulating repair mechanisms after SCI.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 420.07/AA12

Topic: C.08.Stroke

Title: Conserved mechanisms underlying MultiStem® cell therapy across neurological injury and disease

Authors: *B. T. LANG, S. A. BUSCH, R. W. MAYS;
Regenerative Med., Athersys, Cleveland, OH

Abstract: Over the past 20 years, off-the-shelf cellular therapies have steadily progressed through pre-clinical and clinical development for the treatment of neurological disease and injury. However, a full understanding of the mechanism of benefit is necessary to optimize therapeutic efficacy. Athersys, is developing MultiStem, a unique bone marrow derived allogeneic cell therapy based upon the proprietary MAPC® cellular technology, for the treatment of multiple disease indications including ischemic stroke. In order to evaluate conserved mechanisms of action underlying MultiStem treatment, Athersys and collaborators performed focused pre-clinical animal studies in models of CNS injury and disease. Based on the summation of these results, we hypothesize that MultiStem infusion alters the innate immune response via interactions with splenocytes. Detailed pre-clinical biodistribution studies have revealed that MultiStem homes primarily to the spleen, reside in the marginal zone and lead to a global decrease in inflammation. MultiStem therapy has been shown to decrease pro-inflammatory cytokines, increase anti-inflammatory cytokines and ultimately modify the splenocyte phenotype. We have observed a consistent Th2 type response following MultiStem infusion, consisting of increased T regulatory cells and “alternatively” activated (M2) macrophage/microglial phenotype across several neurological indications. Further, our recently completed double-blind, randomized, placebo-controlled Phase 2 safety and efficacy trial in

ischemic stroke has provided strong verification of these pre-clinical findings. Intravenous MultiStem treatment between 24-36 hours following ischemic onset not only provided sustained significant functional improvements at 1 year, but also significantly reduced both global pro-inflammatory cytokine levels and circulating T cells in the blood. Our studies have identified conserved mechanisms underlying immune-modulation leading to neuroprotection and ultimately recovery following MultiStem therapy.

Disclosures: **B.T. Lang:** A. Employment/Salary (full or part-time): Athersys. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys. **S.A. Busch:** A. Employment/Salary (full or part-time): Athesys. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys. **R.W. Mays:** A. Employment/Salary (full or part-time): Athersys. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.08/AA13

Topic: C.09. Brain Injury and Trauma

Support: HRF-201512-012

Title: N-acetyl-l-cysteine reduces hypoglycemia-induced hippocampal neuronal death

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Abstract: Type 1 diabetic patients who attempt tight control of blood glucose levels via insulin or other blood glucose reducing agents are frequently at risk of experiencing severe hypoglycemia. Severe hypoglycemia, which occurs if blood glucose levels fall below 1mM (18mg/dL), can lead to seizures, loss of consciousness and death. Hypoglycemic neuronal cell death is not a simple result of low glucose supply to the brain, but instead results from a cell death process that is initiated by the reintroduction of glucose after 10-30 minutes period of glucose deprivation. Glucose reperfusion after severe and prolonged hypoglycemia promotes oxidative stress, neuronal death and cognitive impairment. Zinc, a biologically essential element

for brain function, is the second most abundant transition metal in our body following iron. Despite, it's essential and ubiquitous role in neuronal function, excessive zinc release from the presynaptic terminals and translocation into postsynaptic neurons may contribute to neuronal death under several disease conditions, such as prolonged epileptic seizure, stroke, traumatic brain injury and hypoglycemia. Accordingly, zinc chelation or vesicular zinc depletion reduces hypoglycemia-induced neuronal death. N-acetyl-L-cysteine (NAC), restores levels of neuronal glutathione (GSH), a potent antioxidant, by providing a cell permeable source of cysteine. NAC also acts as a zinc chelator that alleviates zinc-induced neuronal death processes in the brain. Thus, we hypothesized that NAC treatment can reduce neuronal cell death, not only by increasing GSH concentration but also via zinc chelation. To test whether NAC can reduce hippocampal neuronal death after severe hypoglycemia, we used an animal model of insulin-induced hypoglycemia and injected NAC (300mg/kg/day, *i.p*) for 1 week after hypoglycemia. Severe hypoglycemia was induced by intraperitoneal injection of human insulin (10 U/kg) and iso-electricity was maintained for 30 minutes. Fluorescence staining using FJB was performed to detect degenerating neurons in the hippocampus. One week after the hypoglycemia, the number of FJB (+) cells was increased compared to sham-operated rats. However, 1-week treatment with NAC reduced the number of FJB (+) cells in the hippocampus after hypoglycemia. To detect oxidative stress, we performed immunofluorescence using the 4HNE antibody. In the present study we found that NAC treatment after hypoglycemia significantly reduced oxidative stress compared to vehicle treated control rats. Therefore, NAC treatment may represent a potential tool for reducing hippocampal neuronal death after severe hypoglycemia.

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

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 420.09/AA14

Topic: C.09. Brain Injury and Trauma

Support: HRF-S-52

Title: Effects of an acetylcholinesterase inhibitor, donepezil, on seizure-induced hippocampal neuronal death

Authors: ***J. JEONG**[1], B. CHOI[1], M. LEE[2], H. CHOI[3], H. SONG[2], S. SUH[1];
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Abstract: Epileptic seizures are short episodes of abnormal brain electrical activity - the presence of recurrent and unprovoked seizures is known as epilepsy. During epileptic seizure, the primary symptoms may include tonic-clonic movement or convulsions, accompanied by a loss of memory that may be short or sustained. Many survivors of severe epilepsy display delayed neuronal death and permanent cognitive impairment. Donepezil is acetylcholinesterase inhibitor that has been shown to be effective treatment agent for Alzheimer's disease, dementia and in ischemic or hypoxic settings. However, the role of donepezil on seizure-induced neuronal death remains untested. Thus, the present study sought to evaluate the therapeutic potential of donepezil treatment on seizure-induced neural injury. To test our hypothesis, we used an animal model of pilocarpine-induced seizure. Temporal lobe epilepsy (TLE) was induced by intraperitoneal (*i.p*) injection of pilocarpine (25 mg/kg) in male rats. Donepezil (2.5 mg/kg/day) was injected by gavage for 3 consecutive days before seizure and Fluoro -Jade B (FJB) staining was performed to determine the degree of hippocampal neuronal death. Counter to our original hypothesis, we found an increased number of FJB positive neurons in the hippocampus of donepezil treated animals, compared to those receiving vehicle alone. These results suggest that donepezil treatment may have detrimental effect on hippocampal neuronal survival after seizure, rather than beneficial effects. Caution is needed for using donepezil and other acetylcholinesterase inhibitors in epileptic patients. Keywords: Epilepsy, pilocarpine, neuron death, donepezil

Disclosures: J. Jeong: None. **B. Choi:** None. **M. Lee:** None. **H. Choi:** None. **H. Song:** None. **S. Suh:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 420.10/AA15

Topic: C.09. Brain Injury and Trauma

Support: HRF-S-52

Title: Protocatechuic acid reduces traumatic brain injury-induced neuronal death

Authors: *S. LEE, B. CHOI, S. SUH;
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Abstract: Traumatic brain injury (TBI) is defined as damage to the brain resulting from external mechanical force, such as rapid acceleration or deceleration, impact, blast waves, or penetration by a projectile. A penetrating head injury occurs when an object pierces the skull and breaches

the dura mater, the outermost membrane surrounding the brain. Neuronal death begins to occur in the hippocampus immediately after the traumatic brain injury. Protocatechuic acid (PCA), one of the main metabolites of complex polyphenols, possesses several biological effects, including anti-apoptotic and anti-inflammatory activity. In addition, it has been reported that PCA exhibits protective effects against oxidative damage. Here, the present study aimed to evaluate the therapeutic potential of PCA on TBI-induced neuronal death. Traumatic brain injury was induced using a controlled cortical impact (CCI) model in rat. PCA (30mg/kg) was injected into the intraperitoneal space immediately after TBI. Neuronal death and oxidative stress were evaluated by Fluoro Jade-B (FJB) staining and 4-hydroxy-2-nonenal (4HNE) immunostaining 24 hours after TBI, respectively. Microglia activation was detected by CD11b immunohistochemistry in the hippocampus 1 week following TBI. The post-treatment with PCA decreased the number of degenerating neurons, oxidative injury and microglial activation in the hippocampus. These results suggest that PCA may have therapeutic potential for reducing traumatic brain injury-induced neuronal death. Keywords: Traumatic brain injury, Protocatechuic acid, Oxidative injury, Microglia

Disclosures: **S. Lee:** None. **B. Choi:** None. **S. Suh:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Program#/Poster#: 420.11/AA16

Topic: C.09. Brain Injury and Trauma

Support: HRF-S-52

Title: Protective effects of potocatechuic acid on seizure-induced neuronal death

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Abstract: An epileptic seizure is a transient episode of abnormal brain activity that disrupts normal brain function and can lead to lasting detrimental effects, including neuronal cell death. Protocatechuic acid (PCA), a type of phenolic acid, is a major metabolite of antioxidant polyphenols found in green tea. It has various effects on normal and cancer cells, both in vitro and in vivo and has been shown to have antioxidant and anti-inflammatory activity in vitro. PCA also reduces myocardial infarct size through inhibition of inflammatory response and platelet

aggregation. PCA is also reported to increase cell proliferation and inhibited apoptosis of neural stem cells. However, the effect of PCA on seizure-induced neuronal death in the hippocampus has not been evaluated. Thus, we tested the potential therapeutic effects of PCA on seizure-induced neuronal death in an animal model of pilocarpine-induced seizure, a common model for epilepsy. Seizure was induced by intraperitoneal (i.p) injection of pilocarpine (25 mg/kg) in adult male rats and PCA (30 mg/kg) was injected into the intraperitoneal space for three consecutive days after seizure onset. Neuronal injury and oxidative stress were evaluated at 3-day post-seizure. To confirm whether PCA increases neuronal survival and reduced oxidative injury in the hippocampus, we performed Fluoro-Jade-B (FJB) staining to detect neuronal death and 4-hydroxynonenal (4HNE) staining to detect oxidative stress after seizure. In the present study we found that PCA treatment reduced neuronal death and oxidative stress in the hippocampus, compared to vehicle-treated controls. Therefore, the present study demonstrates that PCA has therapeutic potential for preventing hippocampal neuronal death after epileptic seizure.
Keywords: Epilepsy, pilocarpine, neuron death, protocatechuic acid, microglia, inflammation, oxidative stress

Disclosures: **S. Lee:** None. **B. Choi:** None. **M. Lee:** None. **H. Choi:** None. **H. Song:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Support: the International Foundation for Research in Paraplegia (IRP)

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Title: Sphingosine 1-phosphate receptor 1 regulates retinal ganglion cell survival and axonal regeneration after optic nerve trauma

Authors: ***V. E. PERNET**[1], D. DALKARA[2], S. JOLY[1];
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Abstract: Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid involved in major physiological and pathophysiological processes such as angiogenesis, inflammation, tumorigenesis and neuronal development. In the present study, we used a classical optic nerve injury model in C57BL/6 adult mice to address the function of the S1P-Sphingosine 1-phosphate receptor (S1PR) axis in retinal ganglion cell (RGC) death and axonal growth. After optic nerve lesion, the immunofluorescent signal of S1PR1 on retinal cryosections was lower in axotomized RGCs than in intact mice. Silencing S1PR1 with an adeno-associated virus serotype 2 (AAV2) containing a shRNA specific to S1PR1 (AAV2.shRNA-S1PR1) exacerbated the loss of RGCs induced by optic nerve crush; the survival rate of β3Tubulin-labelled RGCs was decreased by 34% in retinae infected with AAV2.shRNA-S1PR1 compared with AAV2.GFP control treatment. In the optic nerve, S1PR1 silencing had an even more pronounced effect on the reduction of axonal sprouting after lesion; the number of growing axons was reduced by 68% in AAV2.shRNA-S1PR1-injected mice at 100 μm past the injury site relative to control animals. We then addressed the contribution of S1PR1 to the protective and regenerative effects of ciliary neurotrophic factor (CNTF). In this aim, the ShH10.CNTF virus was used to infect Müller glia and sustain CNTF delivery to RGCs. Strikingly, after CNTF stimulation, AAV2-mediated S1PR1 down-regulation caused a 31-% decrease in the density of surviving RGCs relative to control conditions obtained with AAV2.GFP/ShH10.CNTF. The most severe loss of RGCs occurred in the superior quadrant where the density of RGCs was decreased by 58% relative to control retinae. The knock-down of S1PR1 affected RGC survival but had no deleterious effects on axonal regeneration in the injured optic nerve. Together, our results suggest that S1PR1 is involved in the survival of retinal ganglion cells induced by CNTF but is not required for axonal regeneration after optic nerve trauma.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01NS045702

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Title: Sirt1 regulates NG2 expressing progenitor proliferation after white matter injury

Authors: *B. JABLONSKA[1], M. GIERDALSKI[2], T. HAWLEY[3], M. CARTON[2], A. LICHAUCO[2], J. CABRERA-LUQUE[2], T. YUEN[4], D. ROWITCH[5], V. GALLO[2];
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Abstract: Regenerative processes in brain pathologies require generation of distinct neural cell populations from endogenous progenitors. We have previously demonstrated a novel role of Sirt1 in oligodendrocyte progenitor cell (OPC) proliferation in the developing subcortical white matter (WM) induced by perinatal hypoxia (Hx). Sirt1 phosphorylation occurs at residue 47 [Sirt1 (ser47)] as a result of post-translational modifications of Cdk2 and Rb. We also found that Hif1α activation and NAD production occur simultaneously with enhanced OPC proliferation in WM. Here, we demonstrated that Sirt1 distribution after Hx is increased in NG2-expressing progenitors but not in $Mash1^+$ cells. Cellular analysis revealed no Hx-induced changes in the numbers of $Sirt1^+CC1^+$, $Sirt1^+GFAP^+$ and $Sirt1^+Iba1^+$ cells. Also, we further established a causal relationship between Hif1α activation, Sirt1 and Cdk2 in Hx-induced WM injury. Using $Cdk2^{-/-}$ (KO) mice, in which exons 4 and 5 were ablated, we found that Hx increased the number of $Hif1\alpha^+$ cells, as well as Sirt1 $(ser47)^+$ cells in WM. This indicates that Sirt1 is upstream of Cdk2. Furthermore, these data also demonstrate that Hx promotes Sirt1 phosphorylation through kinases other than Cdk2. Consistent with this interpretation, Western Blot analysis confirmed higher levels of Hif1α and Sirt1 (ser 47) after Hx in both WT and KO mice. Further analysis established a link between NAD and Sirt1 activation after Hx. Cultured cells from Nx and Hx WM were incubated in control medium (with EGF + FGF), as well as with NAD or NADH for 24 hours. Immunoprecipitation analysis demonstrated lower levels of acetylated Cdk2 after Hx in control untreated cultures, suggesting increased deacetylation of Cdk2. NADH did not alter the levels of deacetylated Cdk2, which were similar in Nx and Hx cells; however NAD dramatically upregulated the level of deacetylated Cdk2 after Hx, indicating that the increase of NAD observed after Hx promotes Sirt1 activation and Cdk2 deacetylation. Together, these results indicate that Sirt1 is a major Hif1α- and NAD-dependent mediator of Hx-induced NG2-exprssing OPC proliferation in WM, and that Sirt1 directly activates Cdk2.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH/NINDS K08 1K08NS091248

NIH/NINDS RO1 NS 040109

Title: MMP inhibitor SB3CT reduces accumulation of chloride in injured neurons

Authors: *V. I. DZHALA, J. GLYKYS, K. STALEY;
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Abstract: Low intracellular chloride ($[Cl^-]_i$) is an important determinant of post-synaptic $GABA_A$-receptor mediated inhibition. This low $[Cl^-]_i$ is set by the Donnan equilibria between immobile extracellular and cytoplasmic anions in conjunction with homeostatic $[Cl^-]_i$ and volume regulation by the cation-Cl^- co-transporters. However, acute brain injuries including trauma and hypoxia-ischemia alters the Donnan equilibria, which results in cytotoxic edema, elevated $[Cl^-]_i$ and depolarizing GABA responses, leading to epileptogenesis. The mechanism of critical pathological changes causing post-traumatic $[Cl^-]_i$ accumulation remains unknown. One possibility is that injured, gliotic areas have altered extra- and intracellular macromolecular compositions, which may change the balance of Donnan forces that set $[Cl^-]_i$ and the polarity and magnitude of $GABA_A$-receptor activity. Matrix metalloproteinases (MMPs) are involved in the degradation of the extracellular matrix and regulation of neuronal cell death. These calcium (Ca^{2+})-dependent zinc (Zn^{2+})-containing enzymes may be targetable by inhibitors of MMPs. We therefore tested whether SB-3CT, a potent and selective inhibitor of MMP-2 and MMP-9, reduces neuronal $[Cl^-]_i$ accumulation after acute brain injury. Acute hippocampal slices from mice expressing the Cl^- sensitive fluorescent indicator Clomeleon were used as a model of severe traumatic brain injury. Two groups of slices were pre-incubated in control ACSF and in a solution containing 20 μM SB-3CT. High-resolution two-photon fluorescence imaging of neurons expressing Clomeleon was performed 0-200 μm below the slice surface. In both groups of slices, morphological features of acute neuronal trauma associated with the neural shear injury induced during slice preparation included swollen or shrunken cell bodies and dendritic dystrophy and varicosities, mostly in the superficial 0-50 um layer of slices. In the deep layers of the acute slices, the cell bodies of neurons were morphologically preserved and healthy. In two groups of slices, traumatized neurons were characterized by increased $[Cl^-]_i$ in the superficial layers. However, statistical analysis demonstrated significantly lower $[Cl^-]_i$ in the superficial layers (0-50 and 50-100 μm) of slices pre-incubated in SB-SCT. At depth of 100-200 μm, no significant difference was found between two means that enclose that comparison. Our results demonstrate that MMP-2 and MMP-9 inhibitor SB-3CT ameliorates trauma-induced increases in neuronal chloride that could be predicted from effects on Donnan forces. This may exert neuro-protective effects and reduce cytotoxic cerebral edema after brain injury.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH IRTA Fellowship

Title: An integral membrane phospholipid phosphate phosphatase, PLPPR1, overcomes chondroitin sulfate inhibition and promotes plasticity

Authors: *C. AGBAEGBU[1], H. KATAGIRI[1], H. GELLER[1], P. YU[2];
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Abstract: Phospho Lipid Phosphate Phosphatase-Related (PLPPR) proteins (PLPPR1-5), also termed plasticity-related genes (PRGs), are integral membrane proteins characterized by six transmembrane domains and are a subclass of the lipid phosphate phosphatase (LPP) superfamily. A quantitative phosphoproteomic screen designed to determine global phosphorylation changes in neurons in response to chondroitin sulfate proteoglycans (CSPGs), potent regulators of neural plasticity during CNS development and axonal regeneration post-injury, revealed PLPPR1 as a protein whose phosphorylation state was most altered by exogenous CSPG treatment. PLPPR1 has been shown to induce actin-rich membrane protrusions in cell lines and primary neuronal cultures independent of Cdc42 and Arp2/3 signaling. However, its endogenous function is unknown. Here, we report that PLPPR1 expression in neurons overcomes axonal outgrowth inhibition mediated by CSPGs. Furthermore, to elucidate the function of these proteins in vivo, we have generated a knock-out mouse line for PLPPR1. Investigation into the global changes in brain structures using 3D reconstruction revealed enlarged lateral ventricles in the PLPPR1 KO mice. Additionally, using Golgi staining, we discovered reduced cortical and hippocampal dendritic spine density in PLPPR1 KO mice as compared to PLPPR1 WT animals. In summary, our data indicates that PLPPR1 protein may modulate neuronal response to CSPGs and regulate neuronal spine density and therefore may mediate neural plasticity. These studies will further define the functional significance of the interplay between CSPGs and PLPPR proteins as well as contribute, not only, to a more comprehensive understanding of how neural plasticity is modulated but also provide an avenue of investigation to improve therapeutic strategies after injury to the CNS.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Program#/Poster#: 420.16/BB3

Topic: C.09. Brain Injury and Trauma

Support: NIH RO1 NS050465

NIH R01 DK104363

Title: Brain trauma disrupts peripheral metabolism and fructose potentiates these effects

Authors: *Z. YING[1], L. F. F. ROYES[1,4], G. KRISHNA[1], F. GOMEZ-PINILLA[1,2,3];
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Abstract: Traumatic brain injury (TBI) is a complex disorder responsible for high rates of morbidity and mortality. It is known that TBI disrupts brain metabolism and dysfunctional metabolism is an important limitation for coping with secondary injury and the healing process. We have initiated studies to determine the action of TBI on crucial aspects of peripheral metabolism that could, in turn, influence the brain pathology. Here we report that 1week moderate fluid percussion injury (FPI, 2.7 atm) decreased glucose tolerance ($p<0.05$), and levels of plasma insulin had an increasing trend. A subgroup of animal was exposed to fructose consumption for 3 weeks (15% w/v, 3wks) before FPI onset to determine the effects of a metabolic perturbation on TBI pathology. Fructose consumption potentiated the increase in plasma insulin level ($p<0.01$) and the decrease in glucose tolerance ($p<0.05$) in animals exposed to FPI, indicating that fructose exacerbates the disruptive effects of TBI on peripheral glucose regulation. However, consumption of fructose for 3 weeks in intact animals was insufficient to alter peripheral metabolism by itself. The current 3 weeks of fructose consumption also increased escape latency in the Barnes maze test ($p<0.01$) in animals exposed to FPI, but fructose by itself was not sufficient to alter latency in intact animals. We have previously shown that 6 weeks of fructose consumption significantly disrupts memory performance in the Barnes maze in intact rats (Agrawal et al., J Neurophysiol, 2012), and aggravates memory dysfunction after TBI (Agrawal et al, JCBFM, 2016). Our results show that the effects caused by TBI are not limited to the CNS, and that circumstances that compromise brain metabolic homeostasis exacerbate the pathophysiology of TBI. These data emphasize that a short period of fructose consumption poses a risk for the outcome of TBI, and that peripheral pathology is partially dependent on central dysfunction carried by TBI.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 420.17/BB4

Topic: C.09. Brain Injury and Trauma

Title: Validation of lysophosphatidic acid as a target for patients with traumatic brain injury

Authors: J. WOJCIAK[1], N. SABBADINI[2], A. J. MORRIS[3], C. MORGANTI-KOSSMANN[4], A. PÉBAY[5], D. DEUTSCHMAN[2], *R. A. SABBADINI[1];
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Abstract: Lysophosphatidic acid (LPA) is a bioactive lipid which mediates a series of signaling events through specific G-protein coupled LPA receptors (LPARs). LPA is released from activated platelets, astrocytes, choroid plexus cells and microglia and is reported to play major roles in inflammatory processes. In the nervous system, LPA signaling system has been validated as a mediator of neuropathic pain, hydrocephalus. Brain LPA also promotes loss of integrity and opening of the blood brain barrier. Recently published work with traumatic brain injury (TBI) showed significant increase in the concentration of LPA in the cerebrospinal fluid (CSF) of both the Controlled Cortical Impact (CCI) injured rodents and severe TBI patients. Further, following neurotrauma, there is an upregulation of LPARs in both mouse and human brain and spinal cord. Using an established CCI injury model, our two independent intervention studies revealed that treatment with the murine anti-LPA mAb, Lpathomab, reduced edema and hemorrhage normally observed following TBI, and that Lpathomab was neuroprotective, significantly reducing the lesion volume by 40-50% using histological and MRI assessments and in a study where a delayed administration of a single dose of Lpathomab at 2 hours after CCI, resulted in substantial and significant effects on behavioral/functional readouts 10 weeks, suggesting long-term benefits of the treatment. These studies combined unequivocally corroborate the efficacy of the anti-LPA antibody in mitigating neuronal damage and improving behavioral responses in injured mice and suggest that LPA may be a good target for therapeutic intervention in the clinical setting. Consequently, we embarked clinical study where we also validated LPA as a potential therapeutic target in TBI patients by correlating LPA levels in the CSF with clinical scores of Glasgow Coma Scale (GCS), Injury Severity Scale (ISS) and Extended Glasgow Outcome Scale

(GOSE) scores. Levels of LPA in CSF distinguished patient groups having unfavorable (GOSE 1-4) or favorable outcomes (GOSE 5-8) at 6 months post-TBI. This data may identify a TBI patient cohort with higher LPA that would most likely benefit from anti-LPA intervention therapy. Lpath's Lpathomab has shown excellent safety and tolerability profiles in a Phase 1a safety trial with healthy human volunteers, positioning Lpathomab for clinical trials in a targeted TBI patient population where LPA levels are elevated.

Disclosures: **J. Wojciak:** A. Employment/Salary (full or part-time): Lpath, Inc.. **N. Sabbadini:** None. **A.J. Morris:** None. **C. Morganti-Kossmann:** None. **A. Pébay:** None. **D. Deutschman:** None. **R.A. Sabbadini:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lpath, Inc.. F. Consulting Fees (e.g., advisory boards); Lpath, Inc.. Other; Inventor.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH grant R37 HD059288

NIH R01 NS069629

Title: Injury-induced alterations in amygdala e/i balance: synaptic mechanisms

Authors: *H. METHENY[1], C. PALMER[2], A. COHEN[1,2];
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Abstract: Traumatic brain injury (TBI) affects approximately 3.8 million people annually in the U.S, with roughly 5.3 million people, or ≈2% of the U.S. population, suffering persistent TBI-related disability. Individuals that suffer a TBI exhibit an increased likelihood of developing an array of neuropsychological symptoms including anxiety, aggression and depression; which in turn lead to an increase in substance abuse, suicidality, and mortality rate among TBI survivors. Due to its integral role in the expression and regulation of emotion, dysfunction of the amygdala is hypothesized to contribute to the acquisition and/or expression of the neuropsychological symptoms of TBI. Our laboratory has previously shown brain injury-induced circuit-level dysfunction in the amygdala associated with deficits in an amygdala-dependent behavior in a mouse model of mild to moderate TBI (mTBI). In this study, we identify a potential mechanism underlying altered circuit function and connectivity of the amygdala following mTBI. Whole cell

voltage-clamp recordings of glutamatergic spontaneous excitatory post synaptic currents (sEPSCs) onto basolateral amygdala (BLA) pyramidal neurons demonstrated a significant decrease in the frequency of sEPSCs after mTBI. Furthermore, there was a significant decrease in lateral amygdala stimulation evoked excitatory post synaptic currents (eEPSCs), with no changes in evoked inhibitory post synaptic currents (eIPSCs) in BLA pyramidal neurons from brain slices of mTBI animals. Given that the recorded eIPSC is predominately composed of polysynaptic inhibition dependent on activation by glutamatergic neurotransmission, the absence of a reduction in eIPSCs corresponding to the decrease in eEPSCs suggests that brain injury selectively preserves or augments GABAergic inhibition in addition to decreasing glutamatergic excitation. Our findings demonstrate opposing shifts in excitation and inhibition, resulting in a disruption of the excitation/inhibition balance (e/i balance) within the amygdala after mTBI. Investigating the underlying mechanisms of amygdala dysfunction after TBI is crucial to the development of therapeutics aimed at ameliorating neuropsychological symptoms of TBI.

Disclosures: H. Metheny: None. **C. Palmer:** None. **A. Cohen:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.19/BB6

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R37 HD059288

NIH Grant R01 NS069629

Title: Increased dentate gyrus net synaptic efficacy is not due to altered hilar evoked GABAergic transmission following mild traumatic brain injury

Authors: *K. A. FOLWEILER[1,2], H. METHENY[2], B. JOHNSON[2], A. COHEN[2,1];
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Abstract: The dentate gyrus (DG) is a crucial regulator of hippocampal excitability and is thought to act as a filter or gate of cortical input to the hippocampus. Dentate granule cell excitability, and thus the putative gating function, is largely controlled by diverse GABAergic interneuron populations many of which reside in the hilus. Traumatic brain injury is known to cause dentate granule cell hyperexcitability, thereby compromising DG gating. Here, we used voltage-sensitive dye imaging (VSDi) with the voltage sensitive absorbance dye (di-3-ANEPPDHQ) to investigate the spatiotemporal characteristics of stimulus-evoked activity in the

dentate gyrus after injury, and potential alterations in hilar GABAergic interneuron evoked transmission that may contribute to net dentate synaptic efficacy. Hippocampal-entorhinal cortex (HEC) slices were made from male C57/BL6 mice 1 week following lateral fluid percussion injury (LFPI, 1.5 atm) for examination with VSDi. In response to perforant path electrical stimulation, all cell layers of the dentate gyrus (molecular layer, granule cell layer, and hilus) had significantly higher optical signals in slices from animals that underwent LFPI versus sham controls. Additionally, optical signals in area CA3 were increased after LFPI, suggesting enhanced propagation or spread of DG excitability to area CA3 after injury. To isolate hilar interneuron GABAergic transmission, the hilus was electrically stimulated in the presence of glutamatergic antagonists and evoked VSDi optical responses were recorded. The results demonstrate no significant difference in the optical signals between LFPI or sham slices in either the granule cell or molecular layers where these GABAergic interneurons are known to synapse onto granule cells. Overall, these data suggest that DG hyperexcitability does propagate to CA3 and that alterations in hilar-derived, evoked net inhibition do not contribute to this phenotype following experimental traumatic brain injury.

Disclosures: **K.A. Folweiler:** None. **H. Metheny:** None. **B. Johnson:** None. **A. Cohen:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH grant R27 HD059288

NIH grant R01 NS069629

Title: Effects of traumatic brain injury and branched chain amino acid dietary therapy on spatial episodic-like memory

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Abstract: Traumatic brain injury (TBI) can lead to significant cognitive impairment, however the underlying neurological basis for TBI-induced cognitive dysfunction remains unknown. Many lines of research have implicated the hippocampus in the pathophysiology of traumatic brain injury. Furthermore, it has been demonstrated that the hippocampus plays a key role during

episodic memory. However, specific impairments of spatial memory as a component of episodic-like memory after TBI have yet to be demonstrated. Here, we tested the putative effects of TBI on spatial memory (a component of episodic memory) while animals were performing a spatial recognition task. To test the association of TBI and spatial memory dysfunction, we used the lateral Fluid Percussion Injury mouse model of TBI together with a spontaneous novelty exploration task. The task used 3 different interval delays (3 minute, 1 hour and 24 hours) between familiarization and test phase of the task. We found that TBI animals exhibited significantly impaired discrimination only after 1 h and 24 h delays of old versus new spatial locations compared to Sham control animals. To further investigate how brain injury alters spatial memory and to investigate a possible therapy to improve this cognitive dysfunction, we administered dietary therapy consisting of branched chain amino acids (BCAAs) leucine, isoleucine and valine initiated 48 hours after the injury and maintained for 5 days in brain injured mice. We tested spatial memory and quantified ipsilateral hippocampal BCAA levels in animals on BCAA therapy. TBI animals exhibited decreased ipsilateral hippocampal BCCA concentrations compared to levels quantified in Sham control animals and a restoration to normal levels in injured animals on dietary therapy. Injured animals on BCAA therapy also demonstrated an improvement in spatial memory after 1h delay interval in BCAA group compared to TBI animals. These findings indicate that TBI impairs spatial memory after a longer compared to a shorter delay interval, and demonstrated the potential benefit of a possible therapy to improve this cognitive dysfunction.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Program#/Poster#: 420.21/BB8

Topic: C.09. Brain Injury and Trauma

Title: Hyperglycemia increases basal and swelling-induced ROS production in C6 cells

Authors: A. K. EDUAFO[1], N. A. SEITZ[1], G. LI[2], C. A. WAKER[1], *J. E. OLSON[2,1];
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Abstract: Diabetic ketoacidosis (DKA) is a potentially fatal hyperglycemic crisis which can leave survivors with permanent neurological sequelae. Brain edema occurs in 0.5-1% of DKA episodes in children with a mortality rate of 21-24%. In survivors, edema during DKA is associated with decreased mental status and poor clinical outcome. Oxidative stress is seen in a

variety of organs of diabetics and may be a common mechanism of tissue injury in this condition. Additionally, increased production of reactive oxygen species (ROS) occurs in many cell types during osmotic swelling. To explore the potential role of oxidative stress for brain injury in DKA, we quantified levels of ROS production in a rat brain cell line following hyperglycemia and during osmotic swelling. Rat C6 glioma cells were treated with hyperglycemic growth medium (26 mM glucose) for 24 hr. Control cultures received 5.5 mM glucose. Basal ROS production rates were measured with quantitative fluorescence imaging by perfusing cells with isoosmotic (290 mOsm) phosphate-buffered saline (PBS) containing 10 µm dihydroethidium (DHE). After 15 min, the PBS was changed to hypoosmotic (200 mOsm) PBS made by reducing the concentration of NaCl but containing the same DHE concentration. The integrated fluorescence intensity of 5-7 individual cells in each microscope field was quantified using ImageJ. The rate of change in fluorescence was calculated for each cell and these values averaged for each coverslip. For some studies, the NADPH oxidase (Nox) inhibitor, 100 µM diphenyleneiodonium (DPI) or the electron transport inhibitor, 5 µM oligomycin plus 5 µM rotenone were added to the PBS solutions. In parallel experiments basal and electron transport-dependent oxygen consumption was measured in isoosmotic PBS. Basal rates of ROS production increased by 23±3% for cells treated with hyperglycemic medium. This difference in ROS production rate was not seen when oligomycin and rotenone was added to the PBS. The swelling-induced increase in ROS production was more than three-fold higher for hyperglycemic-treated cultures compared with control cultures but was inhibited by DPI for both growth conditions. Basal oxygen consumption decreased from 10.7±0.8 pMoles/(min mg protein) in control cultures to 6.8±0.9 pMoles/(min mg protein) in hyperglycemic-treated cells. Thus, the increase in basal ROS production due to hyperglycemic exposure is due to mitochondrial electron transport despite a decrease in basal respiration. ROS production in swollen cells via Nox also is enhanced following hyperglycemia. Both mechanisms would contribute to oxidative stress and may lead to brain cell injury in DKA patients with brain edema.

Disclosures: **A.K. Eduafo:** None. **N.A. Seitz:** None. **G. Li:** None. **C.A. Waker:** None. **J.E. Olson:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Adelson Medical Research Foundation

Title: Zinc chelation and Klf9 suppression additively promote long distance axon regeneration after optic nerve injury

Authors: *E. F. TRAKHTENBERG[1], Y. LI[1], Q. FENG[1], J. TSO[2], P. A. ROSENBERG[3], J. L. GOLDBERG[5], L. I. BENOWITZ[4];
[1]Neurosurg., [3]Neurol., [4]Neurosurg. and Ophthalmology, [2]Boston Children's Hospital, Harvard Med. Sch., Boston, MA; [5]Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: The inability of neurons in the central nervous system (CNS) to regenerate damaged axons, as well as the limited capacity of undamaged neurons to form compensatory connections, limits recovery from ischemic or traumatic injury in the CNS. Manipulation of various cell-autonomous factors and/or overcoming cell-extrinsic inhibitors of growth in the adult CNS only partially restore regeneration and structural plasticity. Thus, the failure of CNS axons to regenerate after injury remains a major unmet problem. Here, we used a well-established optic nerve crush model in mice to traumatically injure and disrupt the axons of retinal ganglion cells (RGCs) in the optic nerve in order to test combinatorial effects of pro-regenerative treatments. We found that co-treatment with intravitreally injected zinc chelator, TPEN, and shRNA-mediated knockdown (KD) of the Klf9 transcription factor (via intraocular injection of an AAV2), had a much stronger effect than either one alone in promoting long-distance axon regeneration, enabling some axons to regenerate the entire length of the optic nerve in just 2 weeks. The growth factor oncomodulin combined with cAMP had about the same effects as either of the other two treatments, but did not strongly augment their effects. Co-treatment with TPEN and KLF9 KD conferred strong long-term protection on RGCs survival, elevating RGC survival ~7-fold higher than controls after 6 weeks. Furthermore, the effect of co-treatment with TPEN and KLF9 KD on long-distance axon regeneration persisted up to 6 weeks after injury, with some axons regenerating ipsilaterally through the optic chiasm and into the optic tract. Thus, zinc chelation in combination with Klf9 suppression additively promotes long-distance axon regeneration, and holds therapeutic potential for promoting axon regeneration after injury to the optic nerve and perhaps other parts of the CNS.

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Poster

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Title: Adaptaquin is an inhibitor of oxygen-sensing prolyl-hydroxylases that abrogates ATF4-dependent death and improves outcomes from brain injury

Authors: *S. S. KARUPPAGOUNDER[1], I. ALIM[1], M. W. BOURASSA[1], C. C. THINNES[2], T.-L. YEH[2], I. GAZARYAN[1], J. ZHONG[1], S. CHO[1], J. W. CAVE[1], C. J. SCHOFIELD[2], E. SHOHAMI[3], F. COLBOURNE[4], G. COPPOLA[5], R. R. RATAN[1];
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Abstract: Disability or death secondary to brain injury is attributed to blood lysis, liberation of iron and the generation of oxidative stress. Iron chelators bind free iron and prevent neuronal death induced by oxidative stress, but the mechanisms remain unclear. Here we show that the hypoxia-inducible factor prolyl-hydroxylase (HIF PHD) family of iron-dependent oxygen sensors is an effector of iron chelation in abrogating brain hemorrhage. Molecular reduction of the three HIF PHD gene isoforms in the mouse striatum improved functional recovery following brain hemorrhage. Treatment with a low molecular weight, oxyquinoline inhibitor of the HIF PHDs, which we call *Adaptaquin,* reduced neuronal death and behavioral deficits following intracerebral hemorrhage in multiple rodent models. Adaptaquin also improved the cognitive function in a rodent model of traumatic brain injury. Unexpectedly, adaptaquin protects from oxidative death by suppressing ATF4-dependent prodeath gene expression rather than by activate the HIF-dependent prosurvival pathway or bulk iron chelation. Together these findings

identify Adaptaquin as a promising therapeutic agent for enhancing functional recovery following brain injury.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: VA MERIT 1I01RX000684 (S. Gandy)

Title: The mGluR2/3 receptor antagonist BCI-838 reverses anxiety-related behavioral traits in a rat model of blast-related mTBI

Authors: ***G. PEREZ-GARCIA**[1], R. DE GASPERI[2,5], M. GAMA SOSA[2,5], M. LASHOF-SULLIVAN[6], S. AHLERS[6], G. ELDER[3,5], S. GANDY[4,5];
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Abstract: Background: Blast-related mild traumatic brain injury (mTBI) has been a frequent effect of battlefield exposure to improvised explosive devices encountered during the conflicts in Iraq and Afghanistan. Elder and colleagues previously showed that rats subjected to repetitive low-level blast exposure displayed post-traumatic stress disorder (PTSD) related behavioral traits that were present many months after blast exposure (Elder GA, et al. 2012).
One consequence of TBI is reported to be impairment of neurogenesis, raising the possibility that proneurogenic drugs might be effective in modifying the course of latent manifestations resulting from mTBI. We have previously reported that pharmacological inhibition of the mGluR2/3 receptors with BCI-838 leads to pro-cognitive, and anxiolytic/antidepressant effects in rodents (Kim SH, et al. 2014). The aim of this study was to investigate whether administration of BCI-838 could enhance brain function and reverse PTSD-related behaviors in a rat model of blast-related mTBI.
Methods: Rats were subjected to three 75 kPa blast exposures under anesthesia. Starting two weeks after blast exposure they were treated daily with BCI-838 (4 and 10 mg/kg) for 2-months

and tested on a variety of cognitive and anxiety/stress related behavioral tasks.
Results: BCI-838 reversed anxiety in the open field, light/dark escape and zero maze compared to controls.
Conclusions: BCI-838 reversed anxiety-related behaviors in a rat model of blast-related mTBI and could represent a potential pharmacological therapy for veterans suffering from PTSD symptoms following blast-related mTBI.

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Poster

420. Neurochemistry of Injury: Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: DICBR, NIAAA, NIH

Henry M. Jackson Foundation

Title: Changes in the pathology of head injury in mice with differing brain DHA levels

Authors: *A. DESAI[1,2], H. CHEN[2], K. KEVALA[2], H.-Y. KIM[2];
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Abstract: Modern western diets have very low n-3 polyunsaturated fatty acid (PUFA) content and a high n-6PUFA to n-3 PUFA ratio. This is a matter of concern considering that brain levels of docosahexaenoic acid (DHA), a prominent n-3 PUFA, are dependent on the dietary intake of these PUFAs. Previously, we have demonstrated that mice with severe brain DHA depletion had impaired recovery after traumatic brain injury (TBI) compared to DHA-adequate mice. As severe DHA depletion is unlikely to occur in humans, we investigated in this study whether moderate brain DHA depletion affects the recovery outcome. E14 pregnant mice were placed on n-3 PUFA deficient diet and the pups were weaned on n-3 deficient or adequate diet to alter their brain DHA concentration. Controlled cortical Impact and Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA), a newly developed surgery-free model of head injury, were employed to induce TBI in these mice when they were 3.5-4 months old. For repeated head injury, the mice suffered one injury each day for three consecutive days. Mice supplemented with n-3 PUFA had higher level of DHA and lower arachidonic acid in the brain.

TBI caused an increase in glial cell activation along with behavioral deficits. The mice with more brain DHA had less injury-induced glial cell activation. A reduction in white matter injury was also observed in the adequate PUFA group. These changes in pathology were accompanied by a reduction in behavioral deficits in the mice that had more brain DHA as compared mice with less brain DHA. These findings indicate that dietary PUFA may play an important role in protecting the brain from the effects by increasing the resilience of the brain to injury.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grants

VA Grants

Title: Inhibition of cysteine proteases, especially cathepsin B, improves behavioral deficits, pathology and biomarkers in traumatic brain injury and trauma-related animal models

Authors: *G. R. HOOK[1], S. JACOBSEN[2], K. GRABSTEIN[3], M. KINDY[4], V. HOOK[5]; [1]ALSP, Inc., La Jolla, CA; [2]iMed, AstraZeneca Neurosci., Cambridge, MA; [3]Dept. of Chem. Engin., Univ. of Washington, Seattle, WA; [4]Dept. of Pharmaceut. Sci., Univ. of South Florida, Tampa, FL; [5]Dept. of Neurosciences, UCSD, La Jolla, CA

Abstract: Polytrauma patients have elevated plasma cysteine protease activity, especially that of cathepsin B, and higher levels are correlated with increased risk of organ failure and death. Animal studies show that deleting the cathepsin B gene improves outcomes in traumatic brain injury (TBI) models and the trauma-related models of ischemia, chronic inflammatory pain, epilepsy, Alzheimer's disease (AD) and multiple sclerosis (MS). Here, data from various groups including our own are summarized showing that administration of small molecule cysteine protease inhibitors using various treatment regimes provides beneficial outcomes in a variety of TBI and TBI-related animal models. Specifically, inhibitor treatment of TBI animal models improves motor deficits and memory deficits while reducing brain lesion volume, neuronal cell death, apoptotic cell death proteins, including Bax, t-Bid, and mitochondrial cytochrome c, and inflammatory cytokines, including TNF-alpha and IL-1beta. Moreover, TBI-inhibitor studies using cathepsin B deficient animals show that most of the benefits obtained from inhibitor

treatment are due to cathepsin B inhibition. Inhibitor treatment of spinal cord injury models reduces gliosis, GFAP, and DNA fragments. In ischemic models, Inhibitor treatment provides potent neuroprotection by greatly lowering brain neuronal cell death, especially in the hippocampus. Inhibitor treatment of inflammatory pain models reduces pain and inflammatory cytokines, IL-1beta and IL-18. In epilepsy models, inhibitor treatment improves neurological scores and learning ability and eliminates pathological mossy fiber sprouting. Cerebral aneurysms and brain bleeding are major TBI complications and inhibitor treatment reduces motor sensory deficits, aneurysms, tissue loss, and neuronal cell death in those models. Edema is a big issue in TBI and inhibitor treatment of inflammatory edema models reduces edema, inflammatory pain, and necrosis. In transgenic AD mice, which overexpress human APP, inhibitor treatment improves memory deficits, increases long-term potentiation and, in some models, also reduces pathological amyloid plaque and Abeta peptides, including the pernicious pyroglutamate Abeta. MS model treatment results in improved clinical scores, increased age of onset, and reduced spinal cord leukocyte infiltration. Taken together, these data provide compelling evidence that small molecule inhibitors of cysteine proteases may provide significant benefits in the treatment of TBI patients.

Disclosures: **G.R. Hook:** A. Employment/Salary (full or part-time): American Life Science Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals. **S. Jacobsen:** A. Employment/Salary (full or part-time): AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals. **K. Grabstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals. **M. Kindy:** None. **V. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals.

Poster

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Topic: C.09. Brain Injury and Trauma

Support: Guarantors of Brain Fellowship

NIHR Professorship

Title: Apathy following traumatic brain injury increases with damage to the dopamine system

Authors: *P. O. JENKINS[1], N. BOURKE[2], S. DE SIMONI[2], D. J. SHARP[2];
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Abstract: *Background.* Apathy refers to a diminished level of motivation that is not the result of emotional distress, intellectual deficit, or a decreased level of consciousness. It is common following traumatic brain injury (TBI) and causes significant morbidity. Disruption to the dopamine system has been implicated in the causation of apathy in other neurological disorders (Sinha N et al., (2013) Cortex 49(5):1292-303; Mitchell RA et al., (2011) CNS Neurosci Ther 17(5):411-27). Therefore we explored whether disruption to the dopamine system following TBI is related to apathy.
Objective. To investigate whether striatal dopamine transporter (DAT) levels correlate with apathy following TBI.
Methods. 31 subjects who had suffered a TBI at least 6 months previously and had persistent cognitive problems were compared with 15 healthy controls. All subjects underwent an ioflupane (^{123}I) single-photon emission computed tomography (SPECT) scan and completed a Lille Apathy Rating Scale (LARS) (Sockeel et al., (2006) J Neurol Neurosurg Psychiatry 77(5): 579–584) as well as other behavioural questionnaires measuring depression and anxiety (the Hospital anxiety and depression scale), general health (Short form health survey) and fatigue (Visual analogue scale to assess fatigue). For 26 of the TBI subjects, we also obtained the LARS caregiver-based version to obtain an objective measure of apathy. SPECT scans were co-registered to high resolution T1 MRI scans and the Oxford-GSK-Imanova striatal atlas (Tziortzi et al., (2014) Cerebral Cortex 24(5):1165-77) was used to explore the relationship between the limbic, executive and sensorimotor subdivisions of the striatum to the apathy scores.
Results. Patients showed reduced striatal DAT levels compared to controls. Both subjective and objective apathy levels as measured by the LARS self and caregiver questionnaires showed a significant negative correlation with total striatal DAT levels. Within the limbic subdivision of the striatum DAT binding correlated negatively with the LARS caregiver but not self questionnaire. In contrast, DAT levels in the executive and sensorimotor sub-divisions correlated negatively with the LARS self but not the caregiver questionnaire. There was no significant correlation with the other behavioural questionnaires.
Conclusions. Apathy levels after TBI are higher in patients with evidence of reduced striatal dopamine. This suggests that a hypodopaminergic state may cause apathy after TBI and motivates an investigation of whether dopaminergic medications might improve this pervasive problem.

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Poster

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McKnight Brain Research Foundation, Brain and Spinal Cord Injury Research Trust Fund

Title: Age-dependent effects of haptoglobin deletion in neurobehavioral and anatomical outcomes following traumatic brain injury

Authors: *A. V. GLUSHAKOV[1,2], R. A. ARIAS[3], E. TOLOSANO[5], S. DORE[4,2];
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Abstract: Introduction: Cerebral hemorrhages are common features of TBI and their presence are associated with chronic disabilities. Recent clinical and experimental evidenced suggest that haptoglobin (Hp), an endogenous hemoglobin-binding protein most abundant in blood plasma, would be involved in the intrinsic molecular defensive mechanism; though, its role in TBI is poorly understood. The aim of this study was to investigate the effects of haptoglobin deletion on the anatomical and behavioral outcomes in a mouse model of TBI.
Methods: The experiments were performed using controlled cortical impact model (CCI) in wild type (WT) C57BL/6 mice and genetically modified mice lacking Hp gene Hp-/- of the same background in two age cohorts [(2-4 mo old (young adult) and 7 8 mo old (older adult)]. Neurological deficit scores (NDS), activity and circling behavior were assessed at 24 and 48h, and brain pathology at 48h after injury using immuno- and histochemistry.
Results: The data obtained demonstrated age-dependent significant effects on the behavioral and anatomical TBI outcomes and recovery from the injury. Moreover, in the adult cohort, neurological deficits assessed as neurological deficit scores of Hp-/- mice at 24 h were significantly improved as compared to WT (P=0.001); whereas, there were no significant differences in brain pathology between these genotypes. In contrast, in the older adult cohort, Hp-/- mice had significantly larger lesion volumes compared to WT (P=0.0244), but neurological deficits were not significantly different. Immunohistochemistry for ionized calcium-binding adapter molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) did not reveal significant differences in microglial and astrocytic reactivity between Hp-/- and WT mice in both age cohorts.

Conclusions: The results of this study provide clarification on the age-dependent aspects of the brain trauma evident from the differences in anatomical and neurobehavioral outcomes between different age cohorts. Further, this this study provides an insight into the prospective roles of Hp in TBI and other acute brain injuries especially those with complex mechanisms suggesting that systemic Hp might age-dependently interact with the intrinsic defensive mechanisms involved in complex pathways and, thereby, differentially affecting acute brain trauma outcomes.

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Poster

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Support: Center for Neuroscience and Regenerative Medicine

Title: Myelin plasticity supports recovery of nerve conduction velocity after experimental traumatic brain injury

Authors: *R. C. ARMSTRONG, C. M. MARION, N. P. CRAMER, K. L. RADOMSKI, F. YU, Z. GALDZICKI;
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Abstract: Patients with mild TBI (mTBI) often exhibit slow information processing speed which may indicate demyelination in white matter tracts. We used a mouse model of mTBI with traumatic axonal injury in the corpus callosum to examine myelin pathology and plasticity after mTBI. Functional integrity of myelinated axons in the corpus callosum was examined using electrophysiological recording in brain slice preparations from C57BL/6 mice after mTBI. Conduction velocity of the N1 myelinated axon waveform was significantly decreased at 3 days, consistent with demyelination. At 2 and 6 weeks the N1 conduction velocity recovered, suggesting remyelination may contribute to functional recovery. To monitor active remyelination in vivo after mTBI, we generated fluorescent myelin reporter mice. Conditional expression of Cre recombinase was driven in mature oligodendrocytes using PLPCreERT2 mice or in oligodendrocyte progenitor cells using NG2CreERT2 mice. Each driver line was crossed to mTmG reporter mice so that tamoxifen administration induced green fluorescent protein (GFP) labeling of membranes. Analysis of naïve PLPCreERT2:mTmG mice confirmed GFP labeled mature oligodendrocytes and myelin throughout the corpus callosum within 7 days of tamoxifen

administration. NG2CreERT2:mTmG mice were administered tamoxifen on days 2 and 3 post-mTBI to assess the progenitor response and EdU on days 3-7 to label cycling cells. At 7 days post-mTBI, NG2Cre mGFP labeling included cells with multiple short processes that were characteristic of progenitors and morphologically distinct from the myelin sheath labeling in PLPCreERT2:mTmG mice. A third of cycling EdU cells are also NG2Cre fate-labeled, though are only a small proportion of total NG2Cre fate-labeled cells. By 1 month post-mTBI, NG2Cre fate-labeled cells had matured into oligodendrocytes and elaborated myelin along axons. The corpus callosum area labeled with GFP myelin membrane was significantly greater in mTBI mice, relative to sham NG2CreERT2 mice, demonstrating remyelination after mTBI. Overall, mTBI produced demyelination resulting in functional deficits followed by remyelination and recovery.

Disclosures: R.C. Armstrong: None. **C.M. Marion:** None. **N.P. Cramer:** None. **K.L. Radomski:** None. **F. Yu:** None. **Z. Galdzicki:** None.

Poster

420. Neurochemistry of Injury: Therapeutic Strategies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 420.30/BB17

Topic: C.09. Brain Injury and Trauma

Support: NS038079

Sanofi R and D

UCSF/BASIC pilot funds

Title: New therapeutic treatment for traumatic brain injury: targeting p75ntr as an immune-modulator after traumatic brain injury

Authors: *S. LEE[1], A. LIN[2], J. SACRAMENTO[2], N. SINGHAL[2], M.-N. CASTEL[3], B. CANOLLE[3], S. DELBARY-GOSSART[4], B. FERZAZ[3], F. BONO-COLOMBIE[4], J. C. BRESNAHAN[2], M. S. BEATTIE[2];
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Abstract: Traumatic brain injury (TBI) patients often experience systemic immune suppression, causing high risk of infection and sepsis, and leading to poor clinical outcome after trauma. Indeed, TBI patients exhibit infections such as pneumonia, septicaemia, urinary tract infection, all of which result in high mortality. Thus, it is critical to counteract systemic immune

suppression in TBI patients. Although intuitively attractive, dampening systemic inflammation after TBI should be beneficial, however, it often results in very complicated outcomes. Therefore, it is important to broaden our understanding of the complexities of the inflammatory response following TBI to develop new and more specific therapeutic strategies for TBI patients. Myeloid derived suppressor cells (MDSCs) are a heterogeneous immature cell population, which have remarkable ability to suppress T cell function and to regulate innate immune responses by modulating cytokine production by macrophages. Indeed, trauma induces the infiltration of MDSCs into the spleen and subsequently suppresses T cell function, which is probably responsible for poor clinical outcome. However, their involvements in TBI have yet to be explored.
Here, we present for the first time that TBI results in the expansion of the MDSC population in the circulation, and subsequently, the accumulation of MDSCs in the injured brain as well as spleen, as evidenced by flow cytometry and immunostaining. Further, we have seen that TBI reduces the T-cell response signaling in the chronic spleen, which is critically related to immune susceptibility in chronic TBI. However, EVT901, a p75NTR antagonist, blocks the expansion of pro-inflammatory monocytes and MDSCs in the circulation, and inhibits the accumulation of these cells in the injured brain after TBI. Further, flow cytometric analysis indicated that EVT901 restores T cell function by reducing the accumulation of MDSCs in spleen chronically. Together, our data indicates that p75NTR is involved in the differentiation of the immature myeloid cell population into mature monocytes/macrophages in response to LPS and TBI, while EVT901 can regulate this. Altogether, our present data suggest that EVT901 can regulate the innate immune cell response by modulating differentiation of MDSCs and hence chronic immune suppression after TBI.

Disclosures: **S. Lee:** None. **A. Lin:** None. **J. Sacramento:** None. **N. Singhal:** None. **M. Castel:** None. **B. Canolle:** None. **S. Delbary-Gossart:** None. **B. Ferzaz:** None. **F. Bono-Colombie:** None. **J.C. Bresnahan:** None. **M.S. Beattie:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.01/BB18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Doctoral Programme in Molecular Medicine, UEF

Alfred Kordelinin foundation

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Magnus Ehrnooths Foundation

Academy of Finland

Title: Identification of targetable steps to modulate nNOS/NOS1AP(CAPON)-dependent signalling mechanisms

Authors: *L.-L. LI[1,2], M. JAAKKOLA[3], L. T. T. AN[3], L. L. ELO[3], M. J. COURTNEY[2]; [1]A.I. Virtanen Inst., Univ. of Eastern Finland, Kuopio, Finland; [2]Neuronal Signalling Lab,Turku Ctr. for Biotechnology, Univ. of Turku and Abo Akademi Univ., Turku, Finland; [3]Computat. Biomedicine Lab, Turku Ctr. for Biotechnology, Univ. of Turku and Abo Akademi Univ., Turku, Finland

Abstract: The protein NOS1AP (CAPON) is a mediator of signalling pathways downstream of the NMDA receptor/PSD95/nNOS complex. The nos1ap gene is linked to disorders from schizophrenia, post-traumatic stress disorder, and autism to cerebro- and cardiovascular disorders. Interaction between NOS1AP and nNOS regulates small GTPases, iron transport, p38MAPK-linked excitotoxicity, and anxiety. In recent years, NOS1AP-nNOS interaction emerged as a promising drug target in animal models of ischemia and anxiety. Understanding the mechanims of NOS1AP-nNOS interaction and NOS1AP function therefore has broad implications for numerous diseases and drug discovery. We find that the interaction of NOS1AP with nNOS differs radically from the classical PDZ docking previously assumed to be responsible. The NOS1AP PDZ motif has low affinity for the nNOS PDZ domain as measured by multiple methods. In contrast, full-length NOS1AP strongly interacts with nNOS. We find a novel internal region of NOS1AP that we call the ExF motif to be responsible for this discrepancy. Although the C-terminal PDZ motif is neither sufficient nor necessary for binding, it promotes the stability of the nNOS-NOS1AP complex. It therefore potentially affects signal transduction and suggests that functional interaction of nNOS with NOS1AP might be targetable at two distinct sites. We demonstrate that excitotoxic pathways can be regulated, in cortical neuron and organotypic hippocampal slice cultures from rats by peptides derived from ExF motif containing region. To better understand the regulation and function of nNOS-NOS1AP interaction, we developed a mathematical model to predict the generation of the active NOS1AP signalling complex. This model was based on experimentally determined values for the interactions between PSD95, nNOS and NOS1AP. A predictive model such as this may facilitate the identification of key steps in NMDA receptor-NOS1AP signalling most suitable for pharmacological intervention in NOS1AP associated diseases.

Disclosures: L. Li: None. **M. Jaakkola:** None. **L.T.T. An:** None. **L.L. Elo:** None. **M.J. Courtney:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.02/CC1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS04327

NIH Grant DC007905

Title: Endogenous extracellular zinc is neuroprotective against glutamate excitotoxicity mediated via NMDA receptors

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Abstract: Zinc is an endogenous modulator of neurotransmission, notably through its inhibition of NMDA and AMPA receptors (NMDARs and AMPARs), and potentiation of glycine receptors (GlyRs).The majority of free zinc in the brain is loaded into vesicles by the zinc transporter Znt3, and co-released with glutamate. However there is an additional, Znt3 independent extracellular tonic pool of zinc that inhibits extrasynaptic NMDARs and potentiates GlyRs (Anderson et al., PNAS 112:E2705; 2015; Rosello et al., Neurobiol Dis 81:14; 2015). Because extrasynaptic NMDARs receptors are implicated in excitotoxicity (Parsons & Raymond, Neuron 82:279; 2014),we investigated whether the tonic zinc pool limits excitotoxic injury via its inhibition of NMDARs. To quantify tonic zinc levels, we used the extracellular ratiometric zinc probe LZ9. We measured nanomolar levels of extracellular zinc in rat mixed cortical cultures, similar to those measured in fresh brain slices of the dorsal cochlear nucleus. DL-threo-β-benzyloxyaspartate (TBOA; 75 μM), a glutamate transporter inhibitor, induced glutamate toxicity and caused a ~30% decrease in cell viability as measured by LDH cytotoxicity assays ($p < 0.05$). Chelation of endogenous extracellular $Zn2+$ with ZX1 (3 μM), a high-affinity extracellular zinc chelator, increased the toxicity of TBOA treatment, reducing viability to ~50% of control ($p <0.05$). In both cases, the NMDAR antagonist memantine (30 μM) blocked cell death. These results indicate that extracellular tonic zinc is neuroprotective via its inhibition of NMDA receptors.

Disclosures: R.F. Krall: None. **K. Hartnett:** None. **T. Tzounopoulos:** None. **E. Aizenman:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.03/CC2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS065219

Title: Zn^{2+} triggered mitochondrial dysfunction depends upon entry via the mitochondrial Ca^{2+} uniporter

Authors: *S. G. JI[1], J. H. WEISS[2];
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Abstract: Ca^{2+} and Zn^{2+} contribute critically to the induction of acute ischemic neurodegeneration. Zn^{2+} is released at synapses, where it can enter postsynaptic neurons. There are also intracellular pools of Zn^{2+} bound to buffering proteins, which can be released during ischemia. Prior studies have indicated that Zn^{2+} powerfully disrupts mitochondrial function, suggesting that these organelles may be important targets of its effects. Yet, despite this understanding, many pertinent aspects of Zn^{2+} effects on mitochondria remain elusive.
(1) Dependence of Zn^{2+} effects upon its uptake into mitochondria through the mitochondrial Ca^{2+} uniporter (**MCU**). While Zn^{2+} can enter the mitochondria via the MCU, past studies have been limited, in part by the incomplete specificity of MCU antagonists. With the recent identification of the MCU gene and availability of knockouts, we have confirmed the importance of this route and are examining whether mitochondrial Zn^{2+} effects are completely dependent upon uptake through the MCU, or despite diminished uptake, effects can still occur in its absence.
(2) Quantitative relationship between mitochondrial Zn^{2+} loading and its effects. Most prior studies have used high exogenous Zn^{2+} exposures to study the effects of mitochondrial Zn^{2+} accumulation, but the quantitative relation between mitochondrial loading and dysfunction has been little examined. One critical factor is that the amount of Zn^{2+} entry into mitochondria is highly dependent upon the efficiency of its cytosolic buffering, which is normally quite effective at protecting mitochondria from pathological Zn^{2+} loads. Using a combination of exogenous Zn^{2+} exposure and graded disruption of intracellular buffering, we are examining the "dose response" of Zn^{2+} uptake on a range of measures of mitochondrial function.
(3) Opportunities for intervention after Zn^{2+} loading to preserve mitochondrial function. We have found that interventions including Zn^{2+} chelation can diminish mitochondrial dysfunction after cellular Zn^{2+} loads, likely promoting their recovery. We are further examining this and other treatments (including MCU blockade) as well as determining the temporal window of opportunity to intervene after Zn^{2+} loading before dysfunction becomes irreversible.
Despite strong evidence that Zn^{2+} contributes to injury in conditions including ischemia, our

ability to target its deleterious effects for therapeutic benefit is limited. We hope that present *in vitro* studies will suggest new approaches for therapeutic interventions that we plan ultimately to test in more complex models.

Disclosures: S.G. Ji: None. **J.H. Weiss:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.04/CC3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: SISSA intramural grant

Title: Nicotinic receptors neuroprotect rat hypoglossal motoneurons from excitotoxicity evoked by glutamate uptake block

Authors: S. CORSINI, M. TORTORA, *A. NISTRI;
SISSA, Trieste, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease characterized by progressive muscular paralysis due to bulbar and spinal motoneuron degeneration. There is, thus, a strong need to understand the earliest mechanisms of ALS onset and identify new treatments to arrest disease progression. In many ALS patients there are abnormally high levels of glutamate in the CSF because of decreased activity of the excitatory amino acid transporters 2 (EAAT2). This observation supports excitotoxicity as a key mechanism to damage motoneurons in the onset phase of this disease. To mimic this condition, we induced excitotoxicity in an established *in vitro* model of rat brainstem slices using the glutamate uptake blocker, DL-threo-beta-benzyloxyaspartate (TBOA). Brainstem hypoglossal motoneurons (HMs) are highly vulnerable to ALS because of their expression of calcium permeable AMPA receptors and low capacity to buffer intracellular free calcium. Activation of nicotinic acetylcholine receptors (nAChRs) by nicotine is reported to be a neuroprotective mechanism against certain experimental neurodegenerative diseases, neurodevelopmental disorders, and neuropathic pain; for this reason we investigated if nicotine could arrest the excitotoxic damage to HMs, which (together with premotoneurons) express homomeric alpha7 and heteromeric alpha4beta2 nAChRs. On 50% of patch-clamped HMs, sustained network bursting activity was induced by TBOA: bursting cells were most likely those that will later die. Nicotine (1-10 microM) increased inhibitory transmission and depressed synaptic excitation. When applied after TBOA-evoked bursting onset, nicotine, in a dose dependent manner,

decreased or even suppressed bursts. Conversely, nAChR antagonists facilitated bursting emergence in non-burster cells. We next studied how nicotine-mediated burst block could be related to HM survival. Thus, using MTT assay, immunohistochemical and RT-PCR experiments, we observed that prolonged exposure to TBOA (up to 4 h) induced 50 % loss of HMs, impaired energy metabolism, increased intracellular levels of reactive oxygen species (ROS), and upregulated genes connected with the endoplasmic reticulum stress. All these neurotoxic mechanisms were largely decreased or even prevented by coapplication of nicotine. Furthermore, these experiments revealed that nicotine *per se* was not toxic to HMs. Our results demonstrate significant neuroprotection of HMs by nAChR activity that are proposed as possible targets to mitigate the network hyperactivation and cellular distress caused by impairment of glutamate reuptake.

Disclosures: **S. Corsini:** None. **M. Tortora:** None. **A. Nistri:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.05/CC4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH (K08-GM073224),

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March of Dimes Birth Defects Foundation (#12-FY08-167)

Title: General anesthetics regulate autophagy and cell survival via modulating inositol 1,4,5 trisphosphate receptor

Authors: G. LIANG[1], G. REN[1], Y. ZHOU[1], *D. J. JOSEPH[2], B. YANG[1], S. INAN[1], M. YANG[1], A. KING[1], H. WEI[1];
[1]Dept. of Anesthesiol. and Critical Care, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA; [2]Pediatrics Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: General anesthetics provide dual effects of both cytoprotection and cytotoxicity with unclear molecular mechanisms. Here, we provide evidence that the commonly used general

anesthetics isoflurane and propofol control cell survival fate by regulating autophagy via activation of the type 1 inositol trisphosphate receptor Ca^{2+} release channel. An adequate exposure to general anesthetics induces autophagy in the mTOR dependent pathway and may be cytoprotective. In contrast, an excessive exposure to general anesthetics may impair normal autophagy flux and become cytotoxic, explaining anesthetics' exacerbating effect on Alzheimer's disease and high vulnerability of neurotoxicity in the developing brain. Thus, the effects of general anesthetics on apoptosis and autophagy are closely integrated, as both are caused by differential activation of the type 1 inositol trisphosphate receptor, which is involved in anesthetic mediated neurodegeneration in developing brain and in Alzheimer's disease.

Disclosures: **G. Liang:** None. **G. Ren:** None. **Y. Zhou:** None. **D.J. Joseph:** None. **B. Yang:** None. **S. Inan:** None. **M. Yang:** None. **A. King:** None. **H. Wei:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.06/CC5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: UNSW Goldstar Funding 2014

Title: Trpc3 ion channel mediated calcium loading in mouse purkinje neurons

Authors: ***J. PARMAR**[1], A. J. CRAIG[2], M. KLUGMANN[2], G. VON JONQUIERES[2], L. BIRNBAUMER[4], A. J. MOORHOUSE[3], J. M. POWER[2], G. D. HOUSLEY[2];
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Abstract: TRPC3 ion channels are non-selective cation channels that are coupled to mGluR1 in cerebellar Purkinje neurons (PNs) and mediate the slow excitatory post-synaptic current. Here, the genetically encoded calcium indicator GCamP5G was employed to dissect the contribution of TRPC3 ion channels to sustained mGluR-mediated Ca^{2+} loading, by comparing PN Ca^{2+} entry in brain slices from wildtype (WT) and TRPC3 knockout mice. All experiments were conducted with the approval of UNSW Animal Care and Ethics Committee. The cerebellum of TRPC3 null and WT (129SvEv) pups (P3) were injected with a recombinant adeno-associated virus (AAVrh10) driving GCamp5G expression under the control of the 1.1kb CMV enhancer/chicken

beta-actin promotor (CAG). At five to eight weeks, parasagittal cerebellar brain slices (400 μm) were imaged using a confocal microscope (Zeiss 710NLO LSM, 488 nm excitation; 10x/0.3W objective). Recordings were at room temperature with continuous superfusion of carbogen-bubbled bicarbonate buffer-based artificial cerebrospinal fluid (ACSF). MGluR1-mediated TRPC3 activation was achieved by bath application of (*S*)-3,5-Dihydroxyphenylglycine (DHPG,100μM) for 10 minutes. Slices were imaged every 10 seconds. ImageJ (NIH) was used to ascertain mean pixel intensity of individual PN somata. Changes in fluorescence (ΔF/F) were quantified as the fraction of evoked change in GCamP5G Ca^{2+} signal at the onset of DHPG application and 20 seconds before DHPG washout divided by the baseline fluorescence levels 2 minutes prior to DHPG application. These were compared between the TRPC3 null and WT slices. Activation of mGluR1 with DHPG increased PN florescence indicative of an increase in cytosolic Ca^{2+} levels. These GCaMP5G-mediated Ca^{2+} fluorescent signals were detected with a patchy distribution across the medial aspect of the cerebellar cortex. Fluorescent signal typically consisted of an initial peak followed by a sustained elevation in signal. In brain slices from TRPC3 null mice the amplitude of the initial transient Ca^{2+} fluorescent signal was reduced; average TRPC3 null PN $\Delta F/F = 0.47 \pm 0.12$ compared to average WT neuron $\Delta F/F = 1.15 \pm 0.43$; similarly for steady state DHPG responses TRPC3 null = 0.01 ± 0.02 vs WT = 0.35 ± 0.05 (3 brain slices from 2 WT mice and 5 brain slices from 3 TRPC3 null mice; range 3 to 14 PN analysed per field of view). These results support the concept that TRPC3 ion channels may contribute to ischaemic brain injury through sustained mGluR1- mediated Ca^{2+} influx. TRPC3 ion channel blockade in the PNs may thus provide a novel pathway to mitigate the detrimental effects of glutamate excitotoxicity by reducing Ca^{2+} loading of neurons.

Disclosures: J. Parmar: None. **A.J. Craig:** None. **M. Klugmann:** None. **G. Von Jonquieres:** None. **L. Birnbaumer:** None. **A.J. Moorhouse:** None. **J.M. Power:** None. **G.D. Housley:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.07/CC6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS087611 to ANM

Teva Pharmaceuticals

Seahorse Bioscience

Title: Control of excitotoxic injury by mitochondrial glutamate oxidation

Authors: A. S. DIVAKARUNI[1], M. WALLACE[2], A. Y. ANDREYEV[1], I. J. REYNOLDS[4], C. M. METALLO[2], *A. N. MURPHY[3];
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Abstract: Glutamate is the dominant excitatory neurotransmitter in the brain, but under the metabolic stress associated with epilepsy, stroke, traumatic brain injury, and Alzheimer's Disease, extraneuronal glutamate can rise to excitotoxic levels that exacerbates brain injury. The brain is heavily reliant on glucose as metabolic fuel, and slight reductions in glucose availability and oxidation are thought to have a profound impact on healthy brain function. Therefore, it was surprising to find that inhibition of the mitochondrial pyruvate carrier (MPC), an inner membrane transporter that facilitates pyruvate uptake from the cytoplasm into mitochondria, protects neuronal cultures from excitotoxic cell death.
Using a combination of bioenergetics, ^{13}C isotope tracing, and metabolomics, we find that mitochondria of cortical neurons readily use non-glucose substrates to fuel energetics and *de novo* glutamate synthesis. Because of this plasticity, MPC inhibition with UK5099 does not compromise neuronal viability or energy metabolism. Rather, mitochondrial metabolism is rewired to rely more heavily on glutamate to fuel energetics and anaplerosis in response to reduced pyruvate oxidation. The reduction in pyruvate oxidation increases glutamate oxidation and decreases the quantity of glutamate released upon depolarization, limiting the positive-feedback cascade of excitotoxic neuronal injury. Thus, MPC activity can influence the size of the releasable glutamate pool in neurons. The finding links pyruvate metabolism with glutamatergic neurotransmission, and establishes the MPC as a potential therapeutic target to treat neurodegenerative diseases characterized by excitotoxicity.

Disclosures: **A.S. Divakaruni:** F. Consulting Fees (e.g., advisory boards); Seahorse Bioscience. **M. Wallace:** None. **A.Y. Andreyev:** None. **I.J. Reynolds:** None. **C.M. Metallo:** None. **A.N. Murphy:** 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; Teva Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Seahorse Bioscience.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.08/CC7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Combined high throughput screening and high content analysis approaches to neuronal physiology/pathology monitoring provide powerful tools for drug discovery

Authors: *P. KITCHENER, L. PAULHAC, F. SIMON;
Fluofarma, Pessac, France

Abstract: Neuronal physiology is complex and its monitoring *in vitro* can include multiple types of measurements such as calcium homeostasis, cell membrane and mitochondrial membrane potential, survival, neurite network, protein expression and phosphorylation, and synaptogenesis. These parameters are dynamic over different time scales, and measurements rely on different technological approaches. They are often considered separately for drug discovery where relevant measures on a high number of test conditions require the miniaturization to small formats at a reasonable speed. As neuronal physiology is complex, drug screening using only one parameter for primary molecule selection can yield simplistic results when the most useful molecules already approved for CNS have complex profiles of action. We used rat primary cortical neuronal cultures in 384-well plates and combined the use of a high throughput fluorescent imaging plate reader (FlipR), for intracellular calcium and mitochondrial potential assessment, with live content imaging, to kinetically measure neuronal cell death, and endpoint high content imaging of neuritic parameters. Basal intracellular calcium measurements show true basal network properties in the primary cultures with spontaneous calcium oscillations confirming neurons are mature, functional and suitable for other measurements made in parallel. Excitotoxicity exemplified by NMDA receptor subtype stimulation, durably increases intracellular calcium concentrations over minutes, and triggers a loss of mitochondrial potential in less than an hour, followed by an increase of neuronal cytolysis over 12-20 hours, and a reduced viable neuritic network. The 384-well plate format allows for a simultaneous exploration of other glutamate receptor subtypes stimulation and multiple pharmacological treatments of the primary neurons yielding different results. Each treatment triggers different modifications of one, or several, of the measured parameters and a signature for each treatment can be derived by the parallel exploration of multiple parameters. The combination of technological approaches in neurons treated in parallel provides a continuum between measurements of fast short-term effects of test molecules (calcium homeostasis), to slow (mitochondrial membrane potential), and slower (neuronal cytolysis) consecutive events, in a dynamic, kinetic assessment of live cells. These dynamic measurements are complemented, at a relevantly chosen time point with the measurement of fine parameters in the same cells that were monitored by live content imaging, using immunofluorescence labeling with a vast array of possible targets.

Disclosures: **P. Kitchener:** A. Employment/Salary (full or part-time): Fluofarma. **L. Paulhac:** A. Employment/Salary (full or part-time): Fluofarma. **F. Simon:** A. Employment/Salary (full or part-time): Fluofarma.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.09/CC8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Structural disassembly of ATP synthase and its role in mitochondrial permeability transition during glutamate induced neuronal death

Authors: *N. MNATSAKANYAN, H. PARK, J. WU, P. MIRANDA, E. A. JONAS;
Dept. of Intrnl. Med., Yale Univ., New Haven, CT

Abstract: Prolonged exposure of neurons to glutamate triggers intracellular Ca^{+2} overload and induces the opening of the mitochondrial permeability transition pore (mPTP) during neurodegenerative diseases, traumatic brain injury and stroke. In addition to prolonged openings that occur under pathological conditions, transient openings of mPTP serve physiological functions. Despite many studies, the molecular components and the sub-molecular structure of the channel that forms the mPTP remain subject to scientific debate. We demonstrated recently that the membrane embedded c-subunit ring of the mitochondrial ATP synthase forms an uncoupling channel with the biophysical characteristics of mPTP. We show now that the F_1 catalytic portion of the ATP synthase gates the c-subunit channel and that mutations of specific amino acid residues within the channel reduce its conductance. We find that overexpression of wild-type but not low conductance c-subunit channels in primary hippocampal neurons exaggerates neuronal death under glutamate-induced excitotoxicity. We observe significant decrease in ATP synthase F_1 subunit levels during glutamate-induced toxicity, while levels of c-subunit remain unchanged. This suggests that structural disassembly of ATP synthase subdomains unmasks the pore of the c-subunit channel, placing mitochondria at increased risk for permeability transition. We present a novel structure-function model of mPTP gating by highlighting the molecular mechanisms and structural re-arrangements in ATP synthase necessary to open the c-subunit channel. These findings will provide us with increased understanding of the role of mPTP in aging and neurodegeneration.

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Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.10/CC9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Iron overload-induced calcum signals modulates mitochondrial fragmentation in mouse hippocampal neuron cells.

Authors: *D. LEE[1], H. LEE[2], D.-S. LEE[1];
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Abstract: Although iron is necessary for neuronal functions, excessive iron accumulation caused by impairment of iron balance damages to neurons. Neuronal iron accumulation has been observed in several neurodegenerative diseases, such as Alzheimer' disease and Parkinson's diseases. However, the precise mechanisms of iron toxicity in neuron cells are not fully understood. In this study, we investigated the mechanism of iron overload-induced mitochondrial fragmentation in hippocampal HT-22 neuron cells, which were incubated with ferric ammonium citrate (FAC). Mitochondrial fragmentation via dephosphorylation of Drp1(Ser637) and further apoptotic neuronal death were observed in FAC-stimulated HT-22 cells. Furthermore, the levels of intracellular calcium were increased by iron overload. Interestingly, chelation of intracellular calcium rescued mitochondrial fragmentation and neuronal cell death. Iron overload also activated the calcineurin through both Ca^{2+}/calmodulin and Ca^{2+}/calpain pathway. Moreover, the pretreatment of W13 and ALLN, as a calmodulin and calpain inhibitor respectively, attenuated iron overload-induced mitochondrial fragmentation and neuronal cell death. Therefore, these finding suggested that Ca^{2+}-mediated calcineurin signals are a key player in iron-induced neurotoxicity by regulating mitochondrial dynamics. We believed that our results may contribute to the development of novel therapy for neurodegeneration related with iron toxicity.

Disclosures: **D. Lee:** None. **H. Lee:** None. **D. Lee:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.11/CC10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NPRP 6-089-3-021

Title: Intracellular calcium ($[Ca^{2+}]_i$) regulating proteins as targets for chemotherapy in neuroblastoma

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Abstract: Neuroblastoma is a cancer derived from sympathetic nerve cells occurring in infancy. Combinatory treatments are currently available, however, some patients at high-risk of tumor relapse can develop resistance to chemotherapeutic agents, limiting overall survival (<15%, 5-year). $[Ca^{2+}]_i$, a second messenger, mediates the regulation of intracellular signaling pathways related to cell-cycle progression, apoptosis and cell death. We investigate whether the chemotherapeutic treatment of a human neuroblastoma cell line (SH-SY5Y) alters the expression and function of calcium regulating proteins and if their pharmacological manipulation enhances the cytotoxic effect of chemotherapeutics such as cisplatin (CDDP) and topotecan (TOPO). We first employed a $[Ca^{2+}]_i$ sensitive fluorescent dye, Fluo-4-AM, to measure acute and sustained changes in $[Ca^{2+}]_i$ in SH-SY5Ys, following exposure to CDDP or TOPO (10nM-1uM) over 3h. The same concentrations were used to assess their effect on stages of cell death in SH-SY5Y cells via FACS analysis, at 72h. To assess expression levels of calcium regulating proteins, SH-SY5Y cells were stained with fluorescently conjugated antibodies targeting: IP3R1, IP3R3, RyR1, RyR3 and S100α6 proteins. These were imaged via confocal microscopy and quantified via "ImageJ" analysis, +/- 1μM CDDP or 10nM TOPO. Finally, 2-APB or dantrolene (IP3 and RyR receptor antagonists, respectively) were co-administered with CDDP or TOPO to assess their effect on cell viability, via trypan blue staining. Results demonstrate that both chemotherapeutic agents induce a time and concentration-dependent increase in $[Ca^{2+}]_i$ in SH-SY5Y cells. These concentrations promote significant late-stage apoptosis ($p<0.05$) and cell necrosis ($p<0.001$), following sustained exposure to either drug. Moreover, following 72h exposure, equivalent concentrations were also found to increase expression of IP3R3 ($p<0.001$), RyR1 ($p<0.001$) and S100α6 proteins ($p<0.05$) in SH-SY5Y cells. Whilst co-administration of IP3 and RyR antagonists exhibited a trend of enhanced chemotherapeutic cytotoxicity, the effect was only significant for the RyR blocker dantrolene in combination with TOPO in SH-SY5Y

cells ($p<0.001$). Data suggest a role for $[Ca^{2+}]_i$ in cytotoxicity and subsequent cell death of neuroblastoma cells, following exposure to therapeutic agents. The increased expression of calcium regulating proteins, such as RyR, following chemotherapy may warrant pharmacological manipulation to enhance $[Ca^{2+}]_i$ regulated cytotoxicity and subsequent cell death. Potentially, this may benefit high-risk patients with acquired chemotherapy resistance.

Disclosures: D. Busselberg: None. **J.E. McCallum:** None. **E. Varghese:** None. **N. Gopinath:** None. **S. Varghese:** None. **A. Florea:** None.

Poster

421. Excitotoxicity and Calcium

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 421.12/CC11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NPRP 6 - 089 - 3 - 021

Title: Characterization of cross-resistance of cisplatin resistant neuroblastoma cells to other chemotherapeutic agents

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Abstract: Neuroblastoma is a main cause of cancer-related mortality in children. Therapy of this childhood cancer is an immense challenge, in particular concerning effective treatment of high risk neuroblastoma patients who have a poor survival rate, often due to acquired drug resistance to conventional chemotherapy and consecutive tumor relapse. Acquired drug resistance commonly occurs upon chemotherapeutic treatment with standard anticancer drugs such as cisplatin (CDDP). In order to cope with drug resistance, new treatment strategies are required. In this study we investigated whether CDDP resistant neuroblastoma cells show cross-resistance to other anticancer drugs. We focused on the characterization of parental (CDDP sensitive) SH-SY5Y cells in comparison with CDDP resistant SH-SY5Y_R cells (resistant to 1µM). Cytotoxicity tests showed that chronic treatment of SH-SY5Y neuroblastoma cells with increasing concentrations of CDDP (up to 1µM) resulted in acquired CDDP drug resistance. The acquired drug resistance, however, was not specific for CDDP. i.e., we also detected cross-resistance to topotecan (TOPO) and taxol (TAXOL), but not to carboplatin (CBPL). We additionally tested if treatment of parental and CDDP resistant neuroblastoma SH-SY5Y cells

with two epigenetic modifiers, namely the (i) DNA methyltransferase inhibitor 5-aza-deoxycytidine (5-AZA) or (ii) the histone deacetylase inhibitor trichostatin A (TSA), may increase CDDP toxicity in neuroblastoma cells. The experiments demonstrated that TSA was much more effective in triggering cytotoxicity in neuroblastoma cells than 5-AZA.The treatment of 5-AZA alone had only a minor cytotoxic effect on SH-SY5Y neuroblastoma cells even at relative high concentrations (up to 200μM). Combinatory application with CDDP did not increase the effectiveness of chemotherapy. However, SH-SY5Y_R cells showed a cross-resistance to TSA treatment, i.e. TSA had a much weaker cytotoxic effect in the SH-SY5Y_R cells than in the parental SH-SY5Y cells. Overall, we observed cross-resistance to other anticancer drugs and epigenetic modifiers in neuroblastoma cells with acquired CDDP resistance. However, more research is necessary to investigate the mechanistic background of this phenomenon and to identify novel strategies for its modulation in both the experimental and clinical settings.

Disclosures: **A.M. Florea:** None. **G. Reifenberger:** None. **D. Busselberg:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.01/DD1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS Grant 25460277

Title: Cyto-protective and -toxic effects of Cordyceps militaris and cordycepin on rat CNS neurons and PC12 cells

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Abstract: Cordyceps militaris (CM), an herbal mushroom, has believed to be effective for various age-related disorders including the neurodegenerative disease, and its specific major bioactive component, cordycepin (3'-deoxyadenosine), appears more effective than CM. We administered 3% CM (JAPAN SILK BIO R&D CENTER) to Dahl salt-sensitive rats mixed with diet including 8% salt that reduced lifespan of these hypertensive rats, and also cordycepin

(0.1~10μM) to PC12 cells, and analyzed how CNS neurons and PC12 cells were influenced by these biochemicals with morphological, immunocytochemical and biochemical methods. CM increased lifespan (p=0.0001; ~3.3 (control) and ~4 (CM) months) of Dahl rats without reducing blood pressure and brain hemorrhage. CNS neurons including hippocampal pyramidal and Purkinje cells appeared degenerated, distensions of endoplasmic reticulum and Golgi apparatus with denser cytoplasm, in control rats, but normal in CM-treated rats. However, ATP synthase β subunit (β subunit), SIRT3, SOD2, LC3-II/LC3-I ratio, cathepsin D, and phosphorylated (p) AMPK were reduced in expression in CM-treated rats. In PC12 cells, we used naive or undifferentiated (tumorigenic in nature) and NGF-treated differentiated (neuronal in nature) cells. Cordycepin was harmful to undifferentiated PC12 cells, killing them, in a dose-dependent manner, without neurite outgrowth, but beneficial to differentiated cells with enhancing neurite extension and expressions of neuron-specific proteins, such as synaptophysin, MAP2 and neurofilament proteins. In undifferentiated cells, cordycepin enhanced expressions for β subunit, SOD2, LC3-II/LC3-I ratio, cathepsin D and pmTOR, but reduced those for SIRT1, SIRT3, pAMPK and pAkt. In differentiated cells, cordycepin enhanced β subunit, SIRT1, SIRT3, pAMPK and pAkt expressions, but reduced expressions for most of proteins enhanced in undifferentiated cells. CM extracts (0.1~10%) were also administrated to these PC12 cells, showing protein expression changes similar to those for cordycepin. In conclusion, CM may suppress AMPK activity in the brain neurons followed by declined mitochondrial/autophagic functions, retaining less cellular energy consumption associated with neuroprotection and lifespan extension after brain hemorrhage. Cordycepin may kill undifferentiated PC12 cells by activating mTOR through the inhibition of AMPK or Akt, which might be maintained by enhanced mitochondrial/autophagic functions, while it may promote neuroprotection in differentiated cells by the mechanism basically opposite to that for the undifferentiated cell, possibly similar to that proposed for the brain neuron.

Disclosures: **K. Tabata:** None. **S. Ito:** None. **J. Sonoda:** None. **K. Takakura:** None. **K. Nagai:** None. **M. Shiozaki:** None. **M. Shibata:** None. **M. Koike:** None. **Y. Uchiyama:** None. **T. Gotow:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.02/DD2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF Grant 2007–0054931

NRF Grant 2014R1A2A1A11051240

KHIDI Grant HI13C0263

Title: Korean red ginseng and ginsenoside-Rb1/-Rg1 alleviate experimental autoimmune encephalomyelitis by suppressing Th1 and Th17 cells and upregulating regulatory T cells

Authors: *M. LEE[1], M. JANG[2], J. CHOI[2], D. KIM[3], I.-H. CHO[2];
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Abstract: The effects of Korean red ginseng extract (KRGE) on autoimmune disorders of the nervous system are not clear. We investigated whether KRGE has a beneficial effect on acute and chronic experimental autoimmune encephalomyelitis (EAE). Pretreatment (daily from 10 days before immunization with myelin basic protein peptide) with KRGE significantly attenuated clinical signs and loss of body weight and was associated with the suppression of spinal demyelination and glial activation in acute EAE rats, while onset treatment (daily after the appearance of clinical symptoms) did not. The suppressive effect of KRGE corresponded to the messenger RNA (mRNA) expression of proinflammatory cytokines (tumor necrosis factor-α [TNF-α] and interleukin [IL]-1β), chemokines (RANTES, monocyte chemotactic protein-1 [MCP-1], and macrophage inflammatory protein-1α [MIP-1α]), adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and platelet endothelial cell adhesion molecule [PECAM-1]), and inducible nitric oxide synthase in the spinal cord after immunization. Interestingly, in acute EAE rats, pretreatment with KRGE significantly reduced the population of $CD4^+$, $CD4^+$/IFN-γ^+, and $CD4^+$/IL-17^+ T cells in the spinal cord and lymph nodes, corresponding to the downregulation of mRNA expression of IFN-γ, IL-17, and IL-23 in the spinal cord. On the other hand, KRGE pretreatment increased the population of $CD4^+$/$Foxp3^+$ T cells in the spinal cord and lymph nodes of these rats, corresponding to the upregulation of mRNA expression of Foxp3 in the spinal cord. Interestingly, intrathecal pretreatment of rats with ginsenosides (Rg1 and Rb1) significantly decreased behavioral impairment. These results strongly indicate that KRGE has a beneficial effect on the development and progression of EAE by suppressing T helper 1 (Th1) and Th17 T cells and upregulating regulatory T cells. Additionally, pre- and onset treatment with KRGE alleviated neurological impairment of myelin oligodendrocyte glycoprotein35-55-induced mouse model of chronic EAE. These results warrant further investigation of KRGE as preventive or therapeutic strategies for autoimmune disorders, such as multiple sclerosis. (Mol Neurobiol. 2016 April 2016, Volume 53, Issue 3, pp1977-2002.)

Disclosures: **M. Lee:** None. **M. Jang:** None. **J. Choi:** None. **D. Kim:** None. **I. Cho:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

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Program#/Poster#: 422.03/DD3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2011-00503

MRC-2012R1A5A2A28671860

HI12C0035

Title: Involvement of activation of the Nrf2/HO-1 signaling pathway in protection against amyloid beta$_{25-35}$-induced neurotoxicity by sulfuretin

Authors: *S.-H. KWON[1], S.-Y. LEE[2], C.-G. JANG[2];
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Abstract: Sulfuretin, one of the major flavonoid glycosides found in the stem bark of *Albizzia julibrissin* and heartwood of *Rhus verniciflua*, is a known anti-oxidant. We previously demonstrated that sulfuretin inhibits neuronal death via reactive oxygen species (ROS)-dependent mechanisms in cultured cells, although other relevant mechanisms of action of this compound remain largely uncharacterized. As part of our ongoing exploration of the pharmacological actions of sulfuretin, we studied the neuroprotective effects of sulfuretin against amyloid beta (Aβ)-induced neurotoxicity in neuronal cells and investigated the possible mechanisms involved. Specifically, we found in the present study that sulfuretin significantly attenuates the decrease in cell viability, release of lactate dehydrogenase (LDH), and accumulation of ROS associated with Aβ_{25-35}-induced neurotoxicity in neuronal cells. Furthermore, sulfuretin stimulated the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), a downstream target of phosphatidylinositol 3-kinases (PI3K)/Akt. We demonstrated that sulfuretin induces the expression of heme oxygenase-1 (HO-1), an anti-oxidant response gene. Notably, we found that the neuroprotective effects of sulfuretin were diminished by an Nrf2 small interfering RNA (siRNA), the HO-1 inhibitor zinc protoporphyrin IX (ZnPP), as well as the PI3K/Akt inhibitor LY294002. Taken together, these results indicated that sulfuretin protects neuronal cells from Aβ_{25-35}-induced neurotoxicity through activation of Nrf/HO-1 and PI3K/Akt signaling pathways. Our results also indicate that sulfuretin-induced induction of Nrf2-dependent HO-1 expression via the PI3K/Akt signaling pathway has preventive and/or therapeutic potential for the management of Alzheimer's disease (AD).

Disclosures: **S. Kwon:** None. **S. Lee:** None. **C. Jang:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.04/DD4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: ISN Grant Category 1A

Title: Characterisation of dorsolateral prefrontal cortex microstructure following sodium azide induced Alzheimer's disease in rats: kolaviron therapeutic mechanisms

Authors: *O. J. OLAJIDE[1], O. B. AKINOLA[1], S. R. PRICE[2], B. U. ENAIBE[1];
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Abstract: Evolvement of therapeutic targets following neurodegeneration is of major biomedical importance. Kolaviron (Kv) is a biflavonoid complex isolated from seeds of *Garcina kola* - a common oral masticatory agent in Nigeria known to hold medicinal value. Therefore this study evaluated the therapeutic potentials of Kv on cells of the dorsolateral prefrontal cortex (DLPFC), before or after sodium azide (NaN_3)-induced neurodegeneration. Rats were randomly assigned into 5 groups (6 each) and treated daily (orally) as follows: 1 ml of corn-oil (vehicle of Kv, 21 days); Kv only (200 mg/kg) for 21 days; NaN_3 only (20 mg/kg for 5 days); NaN_3 (20 mg/kg for 5 days) followed by Kv (200 mg/kg for 21 days); Kv (200 mg/kg for 21 days) followed by NaN_3 (20 mg/kg for 5 days). After treatments, exploratory behaviour associated with DLPFC function was assessed in the open field test (OFT). Subsequently, rats were sacrificed and perfused transcardially (4% PFA) with brains fixed in accordance of techniques demonstrated. Microscopic anatomy of the DLPFC was examined in histology (H & E), antigen retrieval immunohistochemistry to show astroglia activation (GFAP), neuronal metabolism (NSE), cytoskeleton (NF) and cell cycle dysregulation (p53). Furthermore, we demonstrated iNOS and nNOS by immunofluorescence while western blotting was used to investigate microtubule associated proteins (MAPT & MAP2) and apoptotic regulatory proteins (Bax, BCL-2 and CAD). Subsequently, we quantified the level of G-6-PDH and LDH in the brain tissue homogenate as a measure of neural tissue glucose metabolism patterns. Quantitative analysis was done using ImageJ software and statistical analysis with Graphpad prism (ANOVA) at $p<0.05$. NaN_3 treatment induced neuronal damage, characterized by reduced relative brain weight, pyknosis, karyorrhesis, astrogliosis, axonal/dendritic damages and cytoskeletal dysregulation that subsequently resulted in increased expressions of apoptotic regulatory proteins and behavioural alteration in OFT. These degenerative changes were relatable to the observed iNOS and nNOS upregulations. However, Kv administration attenuated the NaN_3-initiated destructive molecular cascades in the DLPFC of rats through mechanisms that involved inhibition of stressor

molecules and toxic proteins, prevention of stress related biochemical redox, preservation of neuronal integrity, protection of neuronal cytoskeletal framework and subsequently, reduced the level of apoptotic regulatory proteins which led to better OFT outcomes. We concluded that Kv conferred therapeutic benefits on NaN_3-induced neurodegeneration especially when administered before than after the damage.

Disclosures: O.J. Olajide: A. Employment/Salary (full or part-time): University of Ilorin. 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; International Society for Neurochemistry. **O.B. Akinola:** None. **S.R. Price:** None. **B.U. Enaibe:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.05/DD5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The neuroprotective effects and possible mechanism of action of a methanol extract from Asparagus cochinchinensis: *In vitro* and *In vivo* studies.

Authors: *A. JALSRAI[1], T. NUMAKAWA[2], H. KUNUGI[2], D. DIETERICH[3], A. BECKER[3]; [1]Ctr. of Traditional Med., Ulaanbaatar, Mongolia; [2]Dept. of Mental Disorder Research,, Natl. Inst. of Neuroscience, Natl. Ctr. of Neurol. and Psychiatry, Tokyo,, Tokyo, Japan; [3]Fac. of Med., Inst. of Pharmacol. and Toxicology, Magdeburg,, Germany

Abstract: Extracts of Asparagus cochinchinensis (AC) have antitumor, anti-inflammatory, andimmunostimulant effects. The neurobiological mechanisms underlying the effects of AC havenot been sufficiently explored. Thus we performed in vivo and in vitro experiments to furthercharacterize potential therapeutic effects and to clarify the underlying mechanisms.In the tail suspension test immobility time was significantly reduced after administration ofAC which suggests antidepressant activity without effect on body core temperature.Moreover, in animals pretreated with AC infarct size after occlusion of the middle cerebralartery was reduced. In vitro experiments confirmed protective effects. Total saponin obtainedfrom IT significantly inhibited H2O2-induced cell death in cultured cortical neurons. Such asurvival-promoting effect by AC saponins was partially blocked by inhibitors forextracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase Akt (PI3K/Akt)cascades, both of which are known as survival-promoting signaling molecules. Furthermore,phosphorylation of tyrosine phosphatase 2 (Shp2) was induced by AC, and the protectiveeffect of AC was abolished by NSC87877, an inhibitor for

Shp2, suggesting an involvementof Shp2-mediated intracellular signaling in AC saponins. Moreover, AC-induced activation ofpShp2 and ErK1/2 were blocked by NSC87877 inhibitor indicating that activation of thesesignaling pathways was mediated by the Shp-2 signaling pathway.These effects appear to be associated with activation of the Shp-2, ErK1/2 and Akt signalingpathways. Our results suggest that AC has antidepressant-like and neuroprotective (reducinginfarct size) effects and that activation of pShp-2 and pErK1/2 pathways may be involved inthe effects

Disclosures: **A. Jalsrai:** None. **T. Numakawa:** None. **H. Kunugi:** None. **D. Dieterich:** None. **A. Becker:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.06/DD6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT Doctorados No. 222854, Fondo 113111.

Title: Curcumin inhibits the activation and translocation of NF-kB in rat hippocampus after experimental exposure to ozone

Authors: *S. D. NERY-FLORES, M. L. MENDOZA-MAGAÑA, M. A. RAMÍREZ-HERRERA, J. J. RAMÍREZ-VÁZQUEZ, M. M. J. ROMERO-PRADO, A. A. RAMÍREZ-MENDOZA, L. HERNÁNDEZ-HERNÁNDEZ;
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Abstract: *Background:* Ozone (O_3) is one of the major urban air pollutants. The exposure to O_3 leads to the formation of reactive oxygen species (ROS) which activate transcription factors, such as Nuclear Factor Kappa B (NF-κB). In turn, it induces the establishment of inflammatory processes. The hippocampus is highly sensitive to pathophysiological changes such as oxidative stress. NF-kB activation in glial cells (astrocytes and microglia) can indirectly induce neuronal death. NF-kB modulators, as curcumin (CUR), could reduce neuronal damage. The CUR has antioxidant, antiinflammatory, antimicrobial, antiproliferative properties, among others. Its various effects involve the regulation of different transcription factors, proinflammatory cytokines, growth factors and kinases. *Objetive:* This work evaluates the effect of curcumin on activation and translocation of NF-kB in rat hippocampus induced by exposure to ozone. *Methodology:* Fifty male Wistar rats were used (n=5 per group). The control groups were: intact, curcumin and ozone groups. The experimental groups were: the therapeutic group (diet with

CUR after exposure) and the preventive group (diet with CUR before exposure). The intact and curcumin groups were exposed to air stream free of ozone. The remaining groups were exposed daily to O_3 for 4 hours at a concentration of 0.7 ppm for 15 days for acute exposure or 60 days for chronic exposure. At the end of the exposure time, rats were sacrificed and the hippocampus was dissected, homogenate was obtained and the nuclear fraction was separated. To determine the activation and translocation of NF-kB was employed the electrophoretic mobility shift assay (EMSA). The groups were compared with the Kruskal-Wallis test followed by the U Mann-Whitney test. The significance level was set at $p<0.05$. *Results and conclusions:* Ozone control group presented a higher activation of NF-kB compared to the other controls groups ($p<0.001$). Dietary treatment with CUR in the preventive and therapeutic groups decreased translocation and activation of NF-kB ($p<0.001$) in animals exposed to O_3 and this effect occurred in the acute and chronic exposition. These results suggest that therapeutic and preventive dietary administration of CUR is capable of decreasing the activation and translocation of NF-KB after exposure to an oxidizing agent such as O_3.

Disclosures: **S.D. Nery-Flores:** None. **M.L. Mendoza-Magaña:** None. **M.A. Ramírez-Herrera:** None. **J.J. Ramírez-Vázquez:** None. **M.M.J. Romero-Prado:** None. **A.A. Ramírez-Mendoza:** None. **L. Hernández-Hernández:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.07/DD7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2015R1D1A1A01061010

Title: Mitochondrial targets of nobiletin and dieckol in the regulation of neuronal cell survival and death

Authors: J.-H. LEE, *S.-Y. EUN, J. WU, K. AMARSANAA, S.-C. JEON, S.-C. JUNG; Cheju Natl. Univ. Coll Med., Jeju-do, Korea, Republic of

Abstract: Mitochondrial calcium overload is a crucial event in determining the fate of neuronal cell survival and death, which is regulated by a variety of channels, antiporters and pumps, as well as an electron transport chains in the mitochondrial inner membrane. The regulation of mitochondrial calcium overload is considered to be an important strategy to prevent neuronal cell death. Therefore, we investigated here the mechanism of nobiletin and dieckol that inhibit neurotoxic mitochondrial calcium overload during neuronal insults.

Primary cortical neurons were obtained from the cerebral cortices of embryonic day 21 and postnatal 1 day Sprague-Dawley rats. Real-time optic measurements were performed using fluorescent indicators such as TMRE for $\Delta\Psi_m$, Fura-2 AM for cytosolic calcium, Rhod-2 AM for mitochondrial calcium and mitoSox/DCF-DA for mitochondrial reactive oxygen species (ROS) in primary cortical neurons. Mitochondrial calcium, ROS and $\Delta\Psi_m$ was also measured in the isolated mitochondria.The effects of neuroprotection were investigated using MTT cell viability assay.
The results demonstrated that nobiletin, an active compound of citrus peel extract, is able to evoke mild mitochondrial depolarization in both primary cortical neurons and isolated mitochondria, which is able to suppress mitochondrial calcium overload. Moreover, nobiletin-induced mild mitochondrial membrane depolarization was significantly abolished by 5-hydroxydecanoate (5-HD), a specific mitochondrial ATP-sensitive K^+ channel (mtK_{ATP}) blocker. In addition, nobiletin markedly reduced electron transport chain (ETC) complex I inhibitor rotenone-induced mitochondrial ROS generation, suggesting a possibility that complex I might be another mitochondrial target of nobiletin, besides mtK_{ATP}. Dieckol, one of the phlorotannins isolated from marine brown alga *Ecklonia cava*, strongly reduced neurotoxic mitochondrial calcium overload in dose-dependent manner.. The mitochondrial targets of diekol are being further investigated.
We propose that mitochondrial targets such as mtK_{ATP}, ETC complex I and mitochondrial calcium uniporter channels (MCU) may be implicated in the regulation of neuronal cell survival and death. Also, nobiletin and diekol as natural single compounds may be promising neuroprotective agents to prevent neuronal cell death through the regulation of the mitochondrial ion channel/transporter and ETC.

Disclosures: **J. Lee:** None. **S. Eun:** None. **J. Wu:** None. **K. Amarsanaa:** None. **S. Jeon:** None. **S. Jung:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

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Program#/Poster#: 422.08/DD8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective role of Thymoquinone in hippocampal cultures

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Abstract: Thymoquinone (TQ), an active constituent of *Nigella sativa* seeds, possesses anti-inflammatory and neuroprotective properties. TQ is reported to enhance the neuroprotection in different CNS diseases and animal models of neurodegeneration. Aim of the present experiment was explore the efficacy of TQ in neuroprotection against kainic acid excitotoxicity on cultured hippocampal neurons. Primary culture of the hippocampal cells from the 18 days old embryonic hippocampus were grown for 7 days. These cultures were divided into control group (C), kainic acid group (KA), Kainic acid+Thymoquinone group (KA+TQ, n=6 in all groups). Control cultures continued to grow without any further treatment, KA cultures were exposed to media containing kainic acid (0.1μM) for 3hrs and thereafter continued to grow in the normal media for 1week. KA+TQ cultures were exposed to media containing kainic acid (0.1μM) for 3hrs and thereafter continued to grow in the media containing 0.1μM thymoquinone for 1week. Cell proliferation was assessed in all cultures during first 24hrs after commencement of treatment. Cell viability was assessed with MTT essay. Cultures were immunostained for doublecortin (DCX), Beta-3 tubulin (Tuj1) and glial fibrillary acidic protein (GFAP). Number of neurons (DCX and Tuj1positive) and glial cells (GFAP positive) were quantified in all cultures. Cell proliferation and cell viability were significantly increased in KA+TQ group compared to C and KA group ($p<0.01$). Thymoquinone treatment significantly increased the number of neurons in KA+TQ group compared to KA and C group ($p<0.01$). Number of astrocytes were also found to be significantly increased in KA+TQ group compared to KA and C cultures ($p<0.01$). The surviving neurons in the cultures treated with thymoquinone had larger cell bodies and longer processes. We conclude that Thymoquinone protects the neurons from kainic acid exitotoxicity, by increasing glial cell population.

Disclosures: **S.M. Shaikh:** None. **M.S. Rao:** None. **S. Smitha:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.09/DD9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Comparison of the neuroprotective effects of phenolic acid metabolites of berry anthocyanins in cerebellar granule neurons

Authors: *E. IGNOWSKI, A. WINTER, M. BRENNER, M. SNODGRASS, D. LINSEMAN; Biol., Univ. of Denver, Denver, CO

Abstract: Neurodegeneration in specific areas of the brain is the primary cause of many devastating diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's

disease. Oxidative and nitrosative stress, along with excitotoxicity, have been implicated as major underlying factors in neuronal cell death. Nutraceuticals, bioactive compounds found in many fruits, vegetables, spices and herbs, have been shown to have neuroprotective effects in a variety of *in vitro* and *in vivo* disease models. Here, we compared the neuroprotective effects of two structurally similar nutraceuticals, 4-hydroxybenzoic acid (HBA) and protocatechuic acid (PCA), in primary cultures of cerebellar granule neurons (CGNs). These phenolic acids are major metabolites of the anthocyanins, callistephin and kuromanin, respectively, which are found at substantial concentrations in strawberries and blackberries. PCA differs from HBA only by the presence of a single phenolic hydroxyl group which provides PCA with a unique catechol moiety. Both HBA and PCA demonstrated significant protective effects against hydrogen peroxide-induced oxidative stress in CGNs. In contrast, only HBA rescued CGNs from excitotoxicity caused by glutamate. On the other hand, PCA uniquely protected CGNs from nitrosative stress induced by the nitric oxide donor, sodium nitroprusside. Finally, PCA also decreased nitric oxide production and tumor necrosis factor-alpha release elicited by lipopolysaccharide stimulation of BV-2 mouse microglial cells. The data suggest that the catechol moiety of PCA imparts a unique ability to counteract the toxicity of nitric oxide both in neurons and microglia. Due to their distinct neuroprotective profiles, HBA and PCA may be uniquely suited to treat different aspects of neurodegeneration. Future studies of the therapeutic potential of these compounds in preclinical models of neurodegeneration would be most beneficial if they compared the effects of individual and combined treatment with these phenolic acids on disease progression.

Disclosures: **E. Ignowski:** None. **A. Winter:** None. **M. Brenner:** None. **M. Snodgrass:** None. **D. Linseman:** None.

Poster

422. Neuroprotective Mechanisms: Natural Products

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 422.10/DD10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: SFR grant # 562 to D.B.G.

Simmons Student Research Fund Award to M.R.

Title: Curcumin is able to reverse the inhibition of neurotransmitter release by beta amyloid oligomers at choroidal synapses in the embryonic avian eye.

Authors: J. QUINN[1], O. ANDERSON[2], M. RAJSOMBATH[3], *D. GRAY[4];
[1]Biol., [2]Neurosci. & Behavior, [3]Biochem., [4]Simmons Col., Boston, MA

Abstract: Alzheimer's disease (AD) is a prevalent neurodegenerative disease associated with characteristic senile neuritic plaques containing a fibrillar form of beta-amyloid (Ab) peptides. This lab has demonstrated a non-pathological role of a-beta as a possible neuromodulator at avian cholinergic synapses, decreasing acetylcholine (ACh) release by stimulating nitric oxide (NO) synthesis and subsequent synthesis of guanyl cyclase and protein kinase G, eventually leading to decreased exocytosis. Previous studies showed that a nitric acid donor (NOD) (1 uM (3,3-bisaminoethyl-1-hydroxy-2-oxo-1-triazene [NOC-18])) mimics Ab's regulatory effects on ACh release. A-beta's role in AD pathogenesis is also thought to involve NO, which is known to induce formation of inflammatory free radicals. Curcumin, a principle compound of turmeric, is well-described antioxidant known to reduce Ab toxicity. Previously curcumin has been hypothesized to act by disrupting aggregation of Ab peptides into an oligomeric form necessary for toxicity. This study demonstrates curcumin, at ~ 1 uM levels, is able to reverse inhibition of ACh release by Ab oligomers at avian embryonic choroid synaptic terminals, but rules out effects on Ab aggregation since curcumin is also able to reverse inhibition of ACh release by direct NO exposure which is downstream from Ab. Curcumin is not able to reverse the effect of cGMP on ACh release. This suggests that curcumin is acting at the nitric oxide step. Further experiments have determined that curcumin does not prevent the donor from producing NO, but rather blocking the nitric oxide effect. These results demonstrate that curcumin is involved in both the pathological and non-pathological pathways. Further research is underway to determine by what process curcumin can reverse nitric oxide's effects.

Disclosures: J. Quinn: None. **O. Anderson:** None. **M. Rajsombath:** None. **D. Gray:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.01/DD11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Vetarans Affair Merit

R01DK080782

DK50456 (Minnesota Obesity Center Metabolic Studies Core fund)

Title: Ceramide contributes to cognitive impairment and palmitate induced apoptosis in hippocampal cell culture

Authors: *C. WANG[1,2,6], T. A. BUTTERICK[1,2,6], V. MAVANJI[3], C. M. DUFFY[2], M. R. LITTLE[2], E. E. NOBLE[2], J. P. NIXON[1,2], C. J. BILLINGTON[1,4,6], C. M. KOTZ[1,5,6]; [1]Minneapolis VA Hlth. Care Syst., Minneapolis, MN; [2]Food Sci. & Nutr., Univ. of Minnesota, St. Paul, MN; [3]Food Sci. & Nutr., Univ. of Minnesota, St. Pau, MN; [4]Medicine, Food Sci. & Nutr., [5]Neuroscience, Integrative Biol. and Physiology, Food Sci. & Nutr., Univ. of Minnesota, Minneapolis, MN; [6]Minnesota Obesity Ctr., Minneapolis, MN

Abstract: Background: Obesity and consumption of diets high in saturated fats are important risk factors for cognitive impairment. Brain uptake of fatty acids is increased in conditions of metabolic syndrome and aging. In our previous study, feeding rodents a high fat diet impaired their learning. Ceramides, synthesized de novo from saturated palmitate, are increased in brain along with aging and Alzheimer's disease (AD), and an increased ceramide level has been suggested as a biomarker for mild cognitive impairment and AD. We hypothesize that ceramides contribute to hippocampal apoptosis induced by palmitate. Methods: *In vitro*, hippocampal cells were treated with vehicle, myriocin (an inhibitor of de novo ceramide synthesis, 100 nM), palmitate (0.1 mM), or myriocin + palmitate; and measured for cell viability, pro-apoptotic signaling (caspase-3/7 and lipid pro-oxidant 4-HNE), and pro-survival signaling (Bcl-2, Akt and BDNF). Stable isotope [U-^{13}C]-palmitate was used to determine ceramide levels with GC/C/IRMS. We also measured the direct effect of artificial C2- and C6-ceramide (0.1~120 μM) on cell viability. *In vivo*, male Sprague-Dawley rats (nine-month old) were trained in memory tests with a two-way active avoidance paradigm, and given intraperitoneal (IP) injections of C6-ceramide (2 μg/kg) every other day for 28 days during which memory was monitored weekly. The data were analyzed with ANOVA or multivariate assay of general linear model. Results and Conclusions: *In vitro*, palmitate significantly reduced cell viability, Bcl-2, Akt and BDNF, and increased caspase 3/7 activity and 4-HNE; while addition of myriocin attenuated palmitate-induced cell death and caspase 3/7 activity. In parallel, the treatments significantly impacted ceramide (C14~24) production, specifically for C16-Cer which was more than 50% of the total ceramides measured. Palmitate increased C16-Cer by 84.8%, and 70% of the synthesized C16-Cer was from exogenous palmitate. Addition of myriocin greatly reduced C16-Cer by 73.3% vs. that of palmitate alone. Thus, elevated synthesis of ceramides might increase pro-apoptotic and reduce pro-survival signaling; while blockade of ceramide synthesis could reverse the pathways induced by palmitate/ceramides. Directly treating the hippocampal cells with C6-ceramide at 40 μM reduced viability and increased caspase-3/7 activity, supporting an apoptotic role of the ceramide. *In vivo*, learning impairment occurred at three-week of IP ceramide injections, indicated by increased escape latency and failure rates. In conclusion, ceramides contribute to palmitate-induced apoptosis of hippocampal cells and cognitive impairment.

Disclosures: C. Wang: None. **T.A. Butterick:** None. **V. Mavanji:** None. **C.M. Duffy:** None. **M.R. Little:** None. **E.E. Noble:** None. **J.P. Nixon:** None. **C.J. Billington:** None. **C.M. Kotz:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.02/DD12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: TWU Research Enhancement Program

Title: Understanding the effects of TDP43 C-terminal fragments

Authors: *Y. T. KASU, C. S. BROWER;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Despite uncertainty surrounding the exact molecular cause of neurodegeneration, a defining feature is the accumulation and aggregation of neuronal protein fragments resulting from an increase in their production, or a decrease in their removal. Previously, we found that the N-end rule pathway of the ubiquitin-proteasome system is able to degrade specific protein fragments associated with Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). This discovery suggests that defects in the N-end rule pathway may contribute to neurodegeneration. To determine if protein fragments play a causative role in disease, we are examining the ectopic expression of a specific aggregation-prone fragment of the human TDP43 protein ($TDP43^{247-414}$) that was found to be a major constituent in intraneuronal plaques found in patients with ALS and FTLD. $TDP43^{247-414}$ was found to be degraded largely by the N-end rule pathway in a manner that requires the *ATE1*-encoded arginyl transferase. We show that $TDP43^{247-414}$ accumulates in Neuro2a cells and in the brains of mice that lack Ate1. Interestingly, $TDP43^{247-414}$ stabilization partially restored the reduced body weight phenotype seen mice lacking Ate1. Ultimately, these studies will help us to understand the effects of $TDP43^{247-414}$ accumulation on neuronal cell function.

Disclosures: Y.T. Kasu: None. **C.S. Brower:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.03/DD13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Soter S. and Carolyn C. Harbolis Alzheimer's Research Endowment

Title: Chronic iron overload and deficiency in adult male c57b6 mice

Authors: *D. G. PETERS[1], J. R. CONNOR[2], Q. X. YANG[3], M. D. MEADOWCROFT[2]; [1]Neural and Behavioral Sci., [2]Neurosurg., [3]Radiology, The Pennsylvania State Univ. - Col. of Med., Hershey, PA

Abstract: Non-heme iron plays a crucial role for metabolic function within the brain and its regulation is tightly controlled. Increased brain iron levels are unique to humans, are two- to four-fold higher than murine brain tissue, and increase with age. This suggests that iron plays an associative role with the aging process and may influence the progression of neurodegenerative disease through oxidative stress, inflammation, and cell death. Conversely, iron deficiency dramatically impairs brain growth during development, but the impact of iron deficiency on the brain during adulthood has not been evaluated. The goal of this study was to evaluate chronic iron overload and deficiency in a C57B6 mouse model to assess how iron dyshomeostasis impacts neural response, brain morphology, and memory. We modeled iron load with four different diets over the course of 12 months beginning at 10 weeks old: Iron deficiency (2-3ppm Fe), Normal Control (200ppm Fe), 0.1% lipophilic TMH-Ferrocene (TMHF), and 0.5% TMHF. Longitudinal magnetic resonance image (MRI) metrics, plasma collection, and behavioral testing were acquired at three-month intervals. Brain morphology and proton relaxometry (R_2) alterations associated with dietary iron overloading were assessed. At study end, liver and segmented brain regions were collected to measure iron concentration, iron storage protein dynamics, and immuno-histological evaluation. Brain R_2 increased in regions known to store iron (substantia nigra and caudo-putamen) in all dietary groups within the first six months and gradually increased in the frontal cortex and the parietal cortex over the preceding six months. The MRI metrics were exacerbated in the ferrocene dietary groups and developed at three months; spreading into the hippocampus, parietal cortex, and cerebellum at 12 months. Deformation based morphometry revealed that there was an expansion of gray matter in the TMHF groups and expansion of white matter in the iron deficient group at 12 months. Gray matter regions were shown to have increased IBA1 and ferritin expression in the TMHF groups. There appeared to be no significant spatial memory differences that trended over time. These data are significant because they support the view that iron overload and deficiency have lasting effects on brain morphology and inflammation into adulthood. The application of this iron-loading model to assess the relationship between neurodegenerative disease processes and brain iron is currently underway.

Disclosures: D.G. Peters: None. **J.R. Connor:** None. **Q.X. Yang:** None. **M.D. Meadowcroft:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.04/DD14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: International Anesthesia Research Society (IARS) Mentored Award GF13062

University of Virginia SOM Research and Development Career Award

Title: Early exposure to general anesthesia impairs the presynaptic machine for neurotransmitter release

Authors: *N. LUNARDI[1], H. P. OSURU[2], A. OKLOPCIC[3], P. DIANA[4], C. DEFREITAS[3], V. JEVTOVIC-TODOROVIC[2];
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Abstract: Background: The release of neurotransmitter in the synaptic cleft is a highly specialized process, achieved through the coordinated actions of numerous proteins that form the so-called pre-synaptic machine for neurotransmitter release. In recent years it has become evident that the modulation of the expression of key release machinery proteins determines essential properties of synaptic transmission at a given synapse, by affecting docking, priming and exocytosis of synaptic vesicles. Our recently published data indicate that exposure to general anesthesia (GA) in the early postnatal period may impair the release of neurotransmitter at developing excitatory synapses, as manifested by a decrease in the number of vesicles docked at the release site in the subiculum of rats five days after the initial exposure.
Hypothesis: We hypothesized that GA may have detrimental effects on the pre-synaptic machine for vesicle release. To test our hypothesis, we focused on three key components of the protein complex for synaptic release: 1) Synapsin, which is essential in spatially segregating vesicles in the reserve pool and releasing them toward the readily releasable pool upon phosphorylation 2) Complexin I, which transforms vesicles into an active "superprimed" state and 3) Synaptotagmin I, which is crucial in triggering fusion of complexin-activated vesicles upon calcium entry into the pre-synaptic terminal.
Methods: We exposed postnatal day 7 (P7) rats to a clinically relevant combination of midazolam, nitrous oxide and isoflurane at the peak of synaptogenesis, and performed western blot assays to quantify the protein level of total synapsin, phospho-synapsin, synaptotagmin I and complexin I in the subiculum of P12 rats.
Results: We found that the levels of synapsin and its active phosphorylated form, phospho-synapsin, are decreased by more than one third in GA-exposed rats compared to sham-controls

(***, P=0.0001 and 0.0008, respectively). Likewise, there was a significant decrease in the level of synaptotagmin I (***, P=0.0006) and complexin I (***, P=0.0007) in experimental rats compared to sham-controls.
Conclusion: Our data provide proof of concept that exposure to GA at the peak of brain development can impair the expression and activity level of key release machinery components, several days after the initial exposure. In view of a substantial body of knowledge indicating persistent cognitive deficits following an early exposure to GA, further studies are warranted to examine the effects of GA-induced impairment of the pre-synaptic machine for neurotransmitter release on developmental synaptic transmission, plasticity and cognitive outcomes.

Disclosures: **N. Lunardi:** None. **H.P. Osuru:** None. **A. Oklopcic:** None. **P. Diana:** None. **C. Defreitas:** None. **V. Jevtovic-Todorovic:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.05/DD15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NCTR/FDA

Title: Inhaled anesthetic sevoflurane-induced up-regulation of pro-apoptosis proteins in infant monkey brains

Authors: ***Q. GU**, F. LIU, S. SARKAR, S. LIU, J. KANUNGO, C. WANG, W. SLIKKER, Jr., M. G. PAULE;
FDA Natl. Ctr. for Toxicological Res., Jefferson, AR

Abstract: It has been shown previously that general anesthesia during early postnatal development can cause cell death in infant animal brains. However, current knowledge remains limited with regard to the molecular mechanisms underlying this phenomenon. To further analyze pathways involved in the adverse effects of general anesthesia on the developing brain at the molecular level, the present study focuses on the expression of pro-apoptotic proteins in the frontal cortex of rhesus monkeys following exposure to sevoflurane, an inhaled anesthetic for the induction and maintenance of general anesthesia. Compared to the control animals which received room air only, exposure to a clinically relevant concentration of sevoflurane (2.5%) for 9 hours increased the level of the pro-apoptotic proteins Bcl2-associated X (BAX) (+87%), caspase 2 (+723%) and caspase 9 (+628%), suggesting enhanced apoptosis signaling. However, not all pro-apoptotic proteins examined thus far showed up-regulation. For example, Bcl-2

homologous antagonist/killer (BAK), another prominent pro-apoptotic protein, displayed undifferentiated expression levels between the control and treated brains, suggesting further that the up-regulation of pro-apoptotic proteins triggered by the sevoflurane treatment was selective. In addition, extracellular-signal-regulated kinase (ERK), an up-stream regulator of the apoptosis process, was also examined. While the total level of ERK remained unchanged, the level of phosphorylated ERK was increased by 65%, indicating enhanced activity of ERK following sevoflurane exposure. These results suggest that sevoflurane-induced cell death in the infant brain involves the over-expression or enhanced activities of a number of specific pro-apoptotic proteins, which eventually lead to cell death. Further molecular analyses of key signaling proteins may help to better understand the molecular mechanisms underlying general anesthesia-induced cell death in the developing brain.

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Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.06/DD16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The University of Texas, Medical School at Houston, the Department of Neurobiology and Anatomy

Title: Levetiracetam mitigates doxorubicin-induced DNA and synaptic damage in neurons

Authors: *J. F. MORUNO MANCHON[1], Y. DABAGHIAN[2,3], N.-E. UZOR[1,4], S. R. KESLER[5], J. S. WEFEL[5], A. S. TSVETKOV[1,4];
[1]Neurobio. and Anat., Univ. of Texas Med. Sch., Houston, TX; [2]The Jan and Dan Duncan Neurolog. Res. Institute, Baylor Col. of Medecine, Houston, TX; [3]Computat. and Applied Mathematics, Rice Univ., Houston, TX; [4]The Univ. of Texas Grad. Sch. of Biomed. Sci., Houston, TX; [5]Neuro-Oncology, M.D. Anderson Cancer Ctr., Houston, TX

Abstract: Neurotoxicity may occur in cancer patients and survivors during or after chemotherapy. Cognitive deficits associated with neurotoxicity can be subtle or disabling and frequently include disturbances in memory, attention, executive function and processing speed. Searching for pathways altered by anti-cancer treatments in cultured primary neurons, we discovered that doxorubicin, a commonly used anti-neoplastic drug, significantly decreased neuronal survival. The drug promoted the formation of DNA double-strand breaks in primary

neurons and reduced synaptic and neurite density. Pretreatment of neurons with levetiracetam, an FDA-approved anti-epileptic drug, enhanced survival of chemotherapy drug-treated neurons, reduced doxorubicin-induced formation of DNA double-strand breaks, and mitigated synaptic and neurite loss. Thus, levetiracetam might be part of a valuable new approach for mitigating synaptic damage and, perhaps, for treating cognitive disturbances in cancer patients and survivors.

Disclosures: J.F. Moruno Manchon: None. **Y. Dabaghian:** None. **N. Uzor:** None. **S.R. Kesler:** None. **J.S. Wefel:** None. **A.S. Tsvetkov:** None.

Poster

423. Neurotoxic Agents

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Program#/Poster#: 423.07/DD17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS015390

NIH Grant AG33620

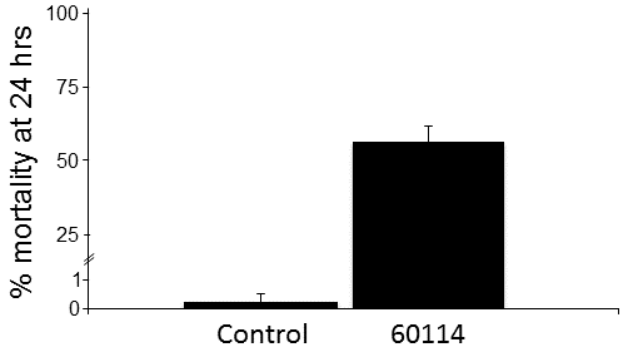
Department of anesthesiology R&D

Title: Mitochondrial complex 1 mutation causes anesthetic-induced mortality in fruit flies

Authors: *M. PEROUANSKY[1], C. LOEWEN[2], Z. OLUFS[3], B. GANETZKY[4];
[1]Dept. of Anesthesiol., Univ. of Wisconsin Madison Dept. of Anesthesiol., Madison, WI; [2]Dept. of Genet., Univ. Wisconsin Madison, Madison, WI; [3]Dept. of Anesthesiol., Univ. of Wisconsin, Madison, WI; [4]Dept. of Genet., Univ. of Wisconsin, University of Wisconsin-Madison, WI

Abstract: Objective: to test whether a loss-of-function mutation in a mitochondrial complex 1 subunit affects sensitivity to volatile general anesthetics (VGAs).
The *Drosophila* NADH dehydrogenase 23 kDa subunit (ND23) is a highly conserved, 'core' subunit of mitochondrial complex 1. Mutations in the human homolog of *ND23* (*Ndufs8*) cause Leigh's syndrome. We discovered a novel, non-synonymous point mutation in *ND23* (*$ND23^{60114}$*) that shortens lifespan and also causes progressive neurodegeneration in flies. We exposed 11-13 day-old *$ND23^{60114}$* flies for two hours to either 2% isoflurane or 3.5% sevoflurane in air and assessed mortality after 24 hrs.
Results: 1 hour after exposure to isoflurane 0.6 ± 0.6% of control flies (wild-type strain) remained immobile, compared to 14.6 ± 3.1% of *$ND23^{60114}$* mutant flies. Twenty-four hours after exposure 0.2 ± 0.2% of control flies were dead, compared to 56 ± 4.5% of mutant flies (see

Figure). Exposure to sevoflurane did not lead to an appreciably increased mortality.
Conclusions: A loss of function mutation in *ND23* renders flies exquisitely sensitive to the toxic effects of isoflurane. Increased sensitivity to the behavioral effects of VGAs has been previously observed in electron transport chain mutants in other model organisms. Humans with analogous mutations are at high risk for adverse effects when exposed to VGAs.
Ref: Kayser EB et al. Anesthesiology 2004; 101: 365-72;
Quintana A et al. PLoS One 2012; 7: e42904;
Niezgoda J et al. Paediatr Anaesth 2013; 23: 785-93



Disclosures: **M. Perouansky:** None. **C. Loewen:** None. **Z. Olufs:** None. **B. Ganetzky:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.08/EE1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG grant EXC 257 NeuroCure

BMBF grant 01 EO 0801

Title: Bortezomib induced apoptosis of adult neural stem cells and post-chemotherapy cognitive impairment in mice

Authors: *P. HUEHNCHEN[1,2,3], W. BOEHMERLE[1,2,3], M. ENDRES[1,2,3,4,5];
[1]Charite Universitaetsmedizin Berlin, Berlin, Germany; [2]Berlin Inst. of Hlth., Berlin, Germany; [3]Cluster of Excellence NeuroCure, Berlin, Germany; [4]Ctr. for Stroke Res. Berlin, Berlin, Germany; [5]German Ctr. for Neurodegenerative Dis., Berlin, Germany

Abstract: Neurotoxic phenomena are among the most common side effects of antineoplastic chemotherapy and present an unmet medical need. Changes of cognitive functions in cancer patients undergoing chemotherapy - often referred to as post-chemotherapy cognitive impairment (PCCI) or "chemobrain" - are diffuse, difficult to attribute to individual drugs and the underlying pathomechanisms are unclear. Bortezomib is a highly potent 26S proteasome inhibitor and mainly used in the treatment of multiple myeloma, but currently under clinical evaluation for the treatment of various solid tumors as well as antibody-mediated autoimmune disorders. The goal of this study is to assess cognitive and histologic changes after bortezomib therapy with a focus on adult hippocampal neurogenesis and evaluate apoptotic pathways in cultured stem and progenitor cells. We used an animal model where adult male C57Bl/6 mice are given a total of 12 intraperitoneal injections of 0.4 mg/kg bodyweight bortezomib (human equivalent dose of 1.2 mg/m^2 body surface) or vehicle over the course of four weeks. Apart from an axonal sensory polyneuropathy, which we previously characterized, the treatment in general was well tolerated. However, bortezomib-treated mice developed distinct visuo-spatial learning and memory deficits as witnessed in the Morris water maze task, while working memory and object recognition were unimpaired. Preliminary histologic analysis revealed a reduced hippocampal cell proliferation after bortezomib therapy that needs to be further characterized. In vitro, brief exposure of murine adult neural stem cells with nanomolar bortezomib concentrations led to severe cytotoxicity, whereas postmitotic mature hippocampal neurons were less susceptible. Apoptosis in neural stem and progenitor cells was mainly mediated by caspase-dependent mechanisms. We hypothesize a disturbance in intracellular calcium homeostasis as initiator of apoptosis, which we are currently further investigating.
In summary, we established a novel animal model to study bortezomib induced PCCI in mice. The preliminary results hint at a link between adult neurogenesis and cognitive dysfunctions due to bortezomib therapy with a caspase-dependent apoptotic mechanism targeting adult neural stem and progenitor cells. Further identification of molecular targets will allow the design of preventive treatment approaches to counteract neurotoxic phenomena.

Disclosures: **P. Huehnchen:** None. **W. Boehmerle:** None. **M. Endres:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.09/EE2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Kuwait University Grant RW01/14

Title: Effect of lead on the expression of metallothionein-3 in the brain of young rats

Authors: *A. RAHMAN[1], K. KHAN[2];
[1]Dept. of Food Sci. and Nutr., Kuwait Univ., Kuwait, Kuwait; [2]Dept. of Anat., Fac. of Medicine, Kuwait Univ., Kuwait, Kuwait

Abstract: Lead (Pb) is a known neurotoxicant which impairs learning and memory. Metallothioneins (MTs) are metal binding proteins that protect cells from heavy metal toxicity. The brain-specific MT-3 has been implicated in the etiology of neurodegeneration. The purpose of this study was to investigate whether exposure to low levels of Pb has an effect on the expression of MT-3 in the rat brain. Wistar rat pups (n=10/group) were exposed to 0.2% Pb-acetate via their dams' drinking water from PND 1 to 21 and directly via drinking water from weaning until PND 30. The control group (n=10) was given regular water. Expression of MT-3 was measured by Western blot (WB) and by immunohistochemistry in various regions of the brain. Western blot analysis of the whole brain lysate from PND21 rats showed two distinct bands; one at ~30kD and one at ~10 kD. Quantitation of these bands revealed that Pb exposure significantly increased MT-3 expression in the brain of PND21 rats. In PND30 rats, only the 30kD band was seen; the 10kD band was not observed. Similar to the PND21 rats, Pb exposure resulted in increased expression of MT-3 in the PND30 rats. From the hippocampal lysate, only the 30kD band was observed and its quantitation revealed that at both PND21 and PND30 the expression of MT-3 was significantly decreased after Pb exposure. Number of immunoreactive neurons in a standardized area of different brain regions were counted and compared between control and Pb-exposed rats. At PND21, more immunoreactive neurons were observed in the cortex and in the CA1, CA2 and CA3 regions of the hippocampus in the Pb-exposed rats compared to control. No effect was seen in the dentate gyrus and the thalamus. At PND30, MT-3 immunoreactive neurons were increased only in the cortex of Pb-exposed rats compared to control, whereas in the thalamus and the CA2 and CA3 regions of the hippocampus fewer MT-3 immunoreactive neurons were observed in the Pb-exposed rats compared to control. CA1 and dentate gurus were largely unaffected. These results suggest that the effect of Pb on MT-3 expression is dependent on the developmental stage of the rats and is brain-region specific.

Disclosures: A. Rahman: None. **K. Khan:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.10/EE3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Abnormal visual function in a mouse model of hemochromatosis with retinal iron loading

Authors: *E. A. MILWARD[1], A. SHAHANDEH[1], D. M. JOHNSTONE[2], A. BRANDLI[3]; [1]The Univ. of Newcastle, Callaghan, Australia; [2]Univ. of Sydney, Sydney, Australia; [3]Univ. of Melbourne, Melbourne, Australia

Abstract: Iron accumulation in the retina may contribute to age-related macular degeneration (AMD) and other serious eye diseases but the mechanisms involved remain unclear. We have assessed molecular and functional changes in the retina of a mouse model of iron loading with defects in the hemochromatosis *Hfe* and transferrin receptor 2 genes on an AKR background (*Hfe*$^{-/-}$x*Tfr2*^{mut}).

We demonstrated substantial retinal iron accumulation by diaminobenzidine-enhanced Perls' stain in the retinal pigment epithelium, photoreceptors, ciliary body and other structures in 9-month-old *Hfe*$^{-/-}$x*Tfr2*^{mut} mice compared to wild-type mice. Immunofluorescence revealed changes in glial fibrillary acidic protein (GFAP), protein kinase α (PKC-α) and glutamine synthase (GS) consistent with retinal stress and degenerative changes.

Ferritin immunofluorescent labelling was increased in *Hfe*$^{-/-}$x*Tfr2*^{mut} mice compared to wild-type mice and gene expression microarrays showed overexpression of iron-related genes, including ferroportin (*Slc40a1*; fold-change 1.394, p=0.03) and transferrin (*Tf*; fold-change 1.843, p=0.002). There were significant expression changes of multiple genes linked to vision disorders including AMD, retinitis pigmentosa, cone-rod dystrophies, congenital stationary night blindness and bradyopsia (all p<0.05).

Retinal function was performed by electroretinography on dark-adapted anesthetized *Hfe*$^{-/-}$x*Tfr2*^{mut} and wildtype AKR mice (n=6/group) between 10 am and 3 pm to reduce circadian fluctuation. The electroretinogram a-wave and b-wave measure photoreceptor and bipolar cell function respectively. After normalization, there were no differences in amplitude or latency of the a-wave (p>0.05) but a reduction of 41% in b-wave amplitude (p=0.03) with 19% delay in the latency (p=0.003). The b-wave is a measure of outer nuclear bipolar cell function and reflects both photoreceptor and synaptic function in the outer plexiform layer of the retina. The changes are consistent with abnormalities involving Müller cells and bipolar cells.

These results provide strong evidence that abnormal retinal iron loading, in association with mutations in the *Hfe* and *Tfr2* genes, can lead to impaired visual function, in conjunction with cellular and molecular changes consistent with retinal stress and degeneration, and may contribute to various important eye diseases.

Disclosures: **E.A. Milward:** None. **A. Shahandeh:** None. **D.M. Johnstone:** None. **A. Brandli:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.11/EE4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effect of an organophosphorus agent on human neuroblastoma cell line SK-N-SH

Authors: *Y. YAMADA[1], K. YAMADA[1], H. SHIRAISHI[2], A. NAMERA[3], Y. ARIMA[3], M. NAGAO[3];
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Abstract: Organophosphates are used in insecticides and as a poison gas in warfare. As acute toxicity, organophosphates are an acetylcholinesterase inhibitor, but the mechanism of action of the chronic toxicity is unclear. Twenty years have passed since a horrifying terrorist attack with sarin gas (isopropyl methyl phosphonofluoridate) at Tokyo. There are many people that have been plagued by severe sequelae and neurological symptoms including deficits in attention and memory, depression, headache, dizziness, insomnia, numbness, ataxia, muscle fatigue, and especially paresthesia in the extremities. We synthesized a sarin-like [bis (isopropyl methyl) phosphonate, BIMP]. To elucidate the mechanism of the chronic toxicity the BIMP toxicity against the human neuroblastoma cell line SK-N-SH and differentiated SK-N-SH by retinoic acid were examined. To differentiate SK-N-SH into neuronal cells, cells were grown in MEMα including 5% FCS together with 40 μM retinoic acid, NGF and BDNF on polylysine coated plates and the medium were replaced every 3 days for 8-30 days. For estimating the toxicity of BIMP, cell viability was analyzed using the conventional MTT reduction assay after incubation for 24 hours. To evaluate the effect of BIMP on the wound healing process,the scratch assay was done using SK-N-SH and differentiated SK-N-SH cells. When the stresses such as toxicity or oxidation are applied to the cells, the endoplasmic reticulum stress causes and increases abnormal proteins. Under the presence of BIMP, the changes of endoplasmic reticulum stress response were examined. The expression level of DNA-damage-inducible transcript (CHOP), which is known as the endoplasmic reticulum stress marker gene were measured by RT-PCR. BIMP inhibited the cell proliferation of SK-N-SH and the IC_{50} value was 44 μM. BIMP showed the inhibitory effect of wound healing on SK-N-SH and differentiated SK-N-SH using a scratch

assay. Expression of major ER related factors CHOP increased after incubation of BIMP for 3 hrs. These results suggested that BIMP induced the chronic neural toxicity by the mechanism related to endoplasmic reticulum stress.

Disclosures: **Y. Yamada:** None. **K. Yamada:** None. **H. Shiraishi:** None. **A. Namera:** None. **Y. Arima:** None. **M. Nagao:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

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Program#/Poster#: 423.12/EE5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant K08-GM073224

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Title: Propofol affects neurodegeneration and neurogenesis by regulation of autophagy via its effects on intracellular calcium homeostasis

Authors: H. QIAO[1,2], Y. LI[3], Z. XU[4], W. LI[1], Z. FU[3], G. LIANG[2], *H. WEI[2];
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Abstract: The effects of most widely used intravenous anesthetic propofol on neurogenesis and associated mechanisms are still not clear. In human cortical neural progenitor cells (NPCs), we investigated the effects of propofol on intracellular calcium homeostasis via activation of the ryanodine and inositol 1,4,5-trisphosphate ($InsP_3$) receptors. We also studied propofol-mediated effects on autophagy, cell survival and neurogenesis. Cell viability was measured by MTT reduction and LDH release assays. Changes on the cytosolic calcium concentration ($[Ca^{2+}]_c$) were evaluated using Fura-2 AM dye, in the presence or absence of the ryanodine receptor antagonist, dantrolene, the $InsP_3$ receptor antagonist, xestospongin C (Xc), intracellular calcium chelator, BAPTA-AM, or the $InsP_3$ production inhibitor, lithium. Autophagy activity was determined by measuring LC3II expression using Western blot. NPC proliferation and

differentiation were also evaluated by bromodeoxyuridine incorporation and immunostaining with neuronal and glial markers. Propofol dose- and time-dependently induced cell damage, most robustly at 200 μM for 24 h. This effect was inhibited by co-treatment with dantrolene, Xc, lithium, BAPTA-AM or autophagy inhibitor 3-MA, but was promoted by autophagy inducer, rapamycin, and autophagy flux inhibitor, bafilamycin. Propofol also dose- and time-dependently elevated the level of the autophagy biomarker LC3II, which was further increased in the presence of bafilomycin but inhibited by dantrolene and Xc. Propofol at 200 μM significantly increased $[Ca^{2+}]_c$, which was partially inhibited by dantrolene, xestospongin C, lithium or BAPTA-AM. Propofol at a clinically relevant concentration (10 μM) stimulated proliferation, which could be abrogated by Xc and dantrolene but proliferation was not affected by either 3-MA or rapamycin. Propofol significantly impaired proliferation at a pharmacological concentration (200 μM), which could be rescued by Xc, dantrolene, 3-MA but was potentiated by rapamycin. Propofol (10 μM) for 24 h increased neuronal differentiation but decreased glial differentiation, while 200 μM propofol for 24 h increased glial differentiation and decreased neuronal differentiation. These dual effects of propofol can be mitigated by co-treatment with either Xc or dantrolene. Propofol dose-dependently induced autophagy activity, cell damage and had dual effects of both promoting and inhibiting neurogenesis in NPCs by differential activation of $InsP_3$ or ryanodine receptor calcium channels. Propofol mediated cell survival or growth is closely associated with its effects on autophagy.

Disclosures: **H. Qiao:** None. **Y. Li:** None. **Z. Xu:** None. **W. Li:** None. **Z. Fu:** None. **G. Liang:** None. **H. Wei:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.13/EE6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Aspartame causes dose dependent hippocampal injury in adult male mice

Authors: ***P. U. NWOHA**[1], A. Y. ONAOLAPO[2];
[1]Obafemi Awolowo Univ., Ile-Ife, Nigeria; [2]Anat., Ladoke Akintola Univ. of Technol., Ogbomoso, Nigeria

Abstract: Aspartame is a widely consumed artificial food sweetener. But opinions differ on its possible adverse neurological effects following consumption. This study therefore investigated the effects of oral administration of aspartame on the histomorphology of the hippocampus in mice. Sixty adult male mice weighing 20 to 22 g, obtained from Animal Holding of Obafemi

Awolowo University were used. Animals received vehicle (distilled water) or one of four doses of aspartame (20, 40, 80 and 160 mg/kg) daily for 28 days via an oral cannula. Animals were properly taken care of, according to accepted ethics and guidelines. At the end of the experiment, the animals were sacrificed and sections of hippocampus processed, and stained with H & E, and cresyl violet, and measured. Data obtained were expressed as mean ± S.E.M and analysed using one-way ANOVA followed by Tukey HSD test. Results showed increased loss of pyramidal and granule neurons, increased glial cells proliferation, and presence of dark staining neurons, with increased dosing. Morphometry revealed significant increase in total cell count and glial cell density, and decrease in cell size and pyramidal cell density at all doses. Granule cell density increased at 20 and 40 mg/kg, and decreased at 80 and 160 mg/kg aspartame consumption. The study concluded that oral administration of aspartame produced dose-dependent hippocampal damage.

Disclosures: P.U. Nwoha: None. **A.Y. Onaolapo:** None.

Poster

423. Neurotoxic Agents

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Program#/Poster#: 423.14/EE7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: HKU Seed Funding for Basic Science Research (201311159171)

Donation from Ms KW Chow

Title: The effects of silica nanoparticles on affective and cognitive behaviors, and on synapse *In vivo* and *In vitro*

Authors: *R. YOU[1], S. S. Y. CHENG[1], C. H. L. HUNG[1,2], Y. S. HO[3], R. C. C. CHANG[1,4,5]; [1]Lab. of Neurodeg. Dis., Sch. Biomed. Sci., HKU, Hong Kong SAR, China; [2]Inst. of Chinese Med. Sciences, Univ. of Macau, Macau SAR, China; [3]Sch. of Nursing, Fac. of Hlth. and Social Sciences, The Hong Kong Polytechnic Univ., Hung Hom, Kowloon, Hong Kong SAR, China, China; [4]Res. Ctr. of Heart, Brain, Hormone and Healthy Aging, LKS Fac. of Medicine, HKU, Hong Kong SAR, China; [5]State Key Lab. of Brain and Cognitive Sciences, HKU, Hong Kong SAR, China

Abstract: Backgrounds Silica nanoparticles (SiO_2-NPs) are typical and major components of mineral dust and many other airborne pollutants in the ambient air. Accumulating evidence show that exposure to SiO_2-NPs may induce neurotoxicity *in vitro* and neuroinflammation. Both of

which are involved in the pathogenesis of neurodegenerative diseases and/or mood dysfunction. However, the effect of SiO_2-NPs on affection and cognition is not known. Moreover, most of the current studies focus on the effects of SiO_2-NPs on neuroinflammation and its contribution to neurotoxicity. The direct effect of SiO_2-NPs on neuron, especially synapse, is poorly known. In the current study, we employed fluorescein isothiocyanate-tagged SiO_2-NPs (FITC-SiO_2-NPs) to investigate the effects of SiO_2-NPs on affection and cognition in mice, and on synapse both *in vivo* and *in vitro*. **Methods** We consecutively exposed 3-month-old male C57BL/6N mice to either vehicle (sterile PBS) or FITC-SiO_2-NPs suspension through intranasal instillation, and subjected the mice to a battery of behavior tests after 1 month and 2 months treatment. We used open field test for locomotor function, elevated plus maze test for anxiety, social interaction test for social activity, accelerated rotarod test for motor function, and novel object recognition (NOR) test and Morris water maze (MWM) test for cognition. Synaptosome fractions were extracted from frontal cortex and hippocampus using Syn-PER reagent. Western-blotting analysis was used to study the protein levels of synaptic proteins in the synaptosomes. FM 1-43 dye was used to study the endocytosis and exocytosis of the synaptosome. In the *in vitro* study, we treated 14-day primary culture of cortical neurons with FITC-SiO_2-NPs for 48 h, and examined the protein levels of synaptic proteins via immunocytochemical analysis and the function of synapse via FM 4-64 FX dye. **Results** We found that FITC-SiO_2-NPs did not affect the locomotor and motor function in mice. However, it decreased social activity after 1-month treatment. 2-month treatment induced anxiety, and impaired short-term memory in NOR test and spatial learning in MWM test. Meanwhile, we found a decrease of synapsin I and an increase of synaptophysin both *in vivo* and *in vitro*. We also found that the exocytosis of frontal cortex synaptosome and cell culture of primary cortical neurons were impaired. **Conclusion** Exposure to FITC-SiO_2-NPs results in mood dysfunction and cognitive impairment in mice, and it may attribute to the structural and functional changes in synapse.

Disclosures: **R. You:** None. **S.S.Y. Cheng:** None. **C.H.L. Hung:** None. **Y.S. Ho:** None. **R.C.C. Chang:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.15/EE8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Shota Rustaveli National Science Foundation Grant FR/533/7-274/14

Title: The effect of arsenic exposure on behavior in rats of various age groups and on pups development

Authors: *T. BIKASHVILI[1], T. LORDKIPANIDZE[2], N. GOGICHAISHVILI[2], N. POCHKHIDZE[2];
[1]I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia; [2]Ilia State Univ., Tbilisi, Georgia

Abstract: Arsenic toxicity is a worldwide health concern as several millions of people are exposed to this toxicant via drinking water, and exposure affects almost every organ system in the body including the brain. Exposure to arsenic induces behavioral and cognitive deficits in both human populations and in rodent models. The effect of arsenic exposure on rat behavior was studied in two different age groups (young and adult). Animals in control groups were given regular water, while the rats from the experimental groups drank the water with final arsenic concentration of 68 mg/L, for three months. Offspring of rats given this dose of arsenic before pregnancy, during pregnancy, and for one month after parturition also were studied. All control and experimental groups were tested: in open field and in elevated plus maze to study the exploratory and anxiety-related behavior; in novel object recognition test to examine the efficacy of memory enhancing compounds; in multi-branched maze to estimate the learning process. The body weight gain abnormalities were observed in all experimental groups. After three months of arsenic exposure the average body weight in young and adult groups and 30-day-old pup's (offspring of arsenic exposure parents) lags behind 20%, 6% and 18%, respectively, in comparison with control animals. The data obtained in this study indicate that arsenic stimulates changes in motor and oriental-searching activity (assessed by the decreased number of lines crossed, rearing and hole reflexes) in the open field test. However, in the experimental groups (especially in the offspring), the animals demonstrated increased levels of anxiety, reflected in the decreased in open arm activity (duration and entries) of elevated plus maze, time spent in the inner circle, inner circle frequency defined by the number of visits into the inner circle of the open field, in increased duration of grooming. Object novelty discrimination index in young and adult control groups, also habituation to the environment (habituation score) in young group was higher in comparison to experimental groups (differences being statistically significant $p<0.05$). Rats from the experimental groups demonstrated impaired cognitive learning: they made more errors and need more time compared to control animals. The difficulty in learning ability was statistically significant ($p<0.05$) in pups, offspring of rats exposed to arsenic. The results suggest that arsenic exposure causes possible health problem not only for parents, but also for the fetus and results in developmental deficits. Acknowledgment. This work was supported by grant from Shota Rustaveli National Science Foundation.

Disclosures: T. Bikashvili: None. **T. Lordkipanidze:** None. **N. Gogichaishvili:** None. **N. Pochkhidze:** None.

Poster

423. Neurotoxic Agents

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.16/EE9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FORMAS

Title: β-N-Methylamino-L-alanine interferes with metabolic pathways related to neurotransmission in human SH-SY5Y neuroblastoma cells as determined by metabolic profiling

Authors: *L. ERSSON[1], M. K. R. ENGSKOG[2], J. HAGLÖF[2], T. ARVIDSSON[2,4], C. PETTERSSON[2], E. BRITTEBO[3];
[2]Div. of Analytical Pharmaceut. Chem., [3]Dept. of Pharmaceut. Sci., [1]Uppsala Univ., Uppsala, Sweden; [4]Med. Product Agency, Uppsala, Sweden

Abstract: β-Methylamino-L-alanine (BMAA) is a non-proteinogenic amino acid produced by ubiquitous organisms such as cyanobacteria, diatoms and dinoflagellates. It is a developmental neurotoxin that induces long-term cognitive deficits as well as an increased ubiquitination, neurodegeneration, astrogliosis, and intracellular fibril formation in the hippocampus of adult rodents following neonatal exposure, whereas it has a low neurotoxic potency in adults rats exposed to BMAA. Recent studies by Cox and coworkers have demonstrated that long-term oral exposure of vervet monkeys to BMAA triggers neurofibrillary tangles formation and amyloid deposits in the brain whereas there were no cognitive changes.
The aim of the present study was to investigate early metabolic effects not related to excitotoxicity of BMAA in differentiated human SH-SY5Y neuroblastoma cells utilizing an analytical platform profiling approach. The cells were exposed to up to 1 mM BMAA during 24 hours and then harvested and snap-frozen prior analysis. Using HILIC-MS and NMR spectroscopy, a plentitude of significantly altered intracellular polar metabolites was detected. Metabolic profiling and multivariate pattern recognition analysis revealed significant perturbations in protein biosynthesis, amino acid metabolism and TCA-cycle. The alterations were preferentially observed in the alanine, aspartate and glutamate metabolism pathways demonstrating that BMAA can interfere with fundamental metabolic pathways related to neurotransmission. In addition, there was a significant enrichment of GABA.
The observed perturbations in amino acid/neurotransmitter metabolism pathways were not related to excitotoxicity or oxidative stress but may be of importance for synaptic plasticity, which in turn could play a role in the neurodegenerative changes induced by BMAA.

Disclosures: L. Ersson: None. **M.K.R. Engskog:** None. **J. Haglöf:** None. **T. Arvidsson:** None. **C. Pettersson:** None. **E. Brittebo:** None.

Poster

423. Neurotoxic Agents

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Program#/Poster#: 423.17/EE10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CAPES

CNPq

FAPESC

PPGBQA-UFSC

PGFAR-UFSC

Title: Depressive-like behavior in adult rats chronically exposed to a glyphosate-based herbicide: involvement of glutamatergic excitotoxicity and oxidative stress

Authors: *D. CATTANI[1], P. A. DE OLIVEIRA[2], R. D. S. PREDIGER[2], E. B. PARISOTTO[1], D. WILHELM FILHO[1], A. Z. PACHECO DE SOUZA[1];
[1]Dept. of Biochem., [2]Dept. of Pharmacol., Federal Univ. of Santa Catarina, Florianópolis, Brazil

Abstract: Epidemiological studies have demonstrated that exposure to pesticides is associated to an increased risk of neurological disorders such as depression and neurodegenerative diseases. Glyphosate [N-(phosphonomethyl)glycine] formulations are the most widely used herbicides worldwide. This study investigated the effects of chronic oral exposure to glyphosate-based herbicide (GBH) on depressive-like and/or anhedonia-like behavior and in the hippocampus of adult male Wistar rats. Female rats were exposed to 1% GBH in drinking water (0.36% or ~ 21.3 mM glyphosate) from gestational day 5 until the offspring was 60 days old. The forced swimming test was used as a model of depressive-like behavior; the locomotor activity was investigated through open field and rotarod tests and sucrose consumption was used as anhedonia-like behavior. For biochemical analysis, hippocampal homogenates were used to evaluate the $^{45}Ca^{2+}$ influx; L-[^{14}C]-glutamate uptake and oxidative stress markers. A depressive-like behavior was observed in GBH-treated rats as demonstrated by the prolonged immobility time during forced swimming test. Moreover, there were no effects on anhedonia-like behavior or locomotor activity in the GBH-treated animals. The ex vivo studies showed a decreased L-[^{14}C]-glutamate uptake and increased $^{45}Ca^{2+}$ influx in hippocampal slices of GBH-treated 60-day old rats, suggesting the induction of glutamatergic excitotoxicity in hippocampus. There was also an increased activity of catalase and glutathione S-transferase indicating a defense reaction against oxidative damage. Taken together, the results demonstrated that chronic exposure to GBH during the development and later on might lead to glutamate excitotoxicity and oxidative

stress in the hippocampus of adult rats. These neurochemical events may contribute, at least in part, to the depressive-like behavior observed in the GBH-treated rats.

Disclosures: **D. Cattani:** None. **P.A. de Oliveira:** None. **R.D.S. Prediger:** None. **E.B. Parisotto:** None. **D. Wilhelm Filho:** None. **A.Z. Pacheco de Souza:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.18/EE11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Assessing drug neurotoxicity and functional mode of action using high-throughput MEA recording from human iPSC neurons combined with multivariate spike train analysis

Authors: *K. JÜGELT, A. STEDER, O. H. U. SCHROEDER, B. M. BADER;
NeuroProof GmbH, Rostock, Germany

Abstract: Identification of neurotoxic effects in drug discovery leads to significant attrition rates, and the lack of suitable in vitro test systems to identify sub-cytotoxic concentrations partly accounts for this. Additionally, the low throughput and high animal costs for presently used brain slice or behavior assays for predicting seizure risk is increasing costs, which is accompanied by the fact that results from rodent tests not always translate into the human situation.
Human stem cell (hiPSC)-based in vitro platforms promise to serve as an alternative to animal in vivo or in situ models. Testing new lead compounds in hiPSC-derived functional neuronal in vitro systems will be physiologically more relevant and further improve current state-of-art by offering higher throughput and higher content. We cultured commercially available hiPSC neurons on multiwell microelectrode arrays which are designed for high-throughput screening. After several weeks in vitro, we tested known neurotoxins such as seizurogenic compounds an analyzed the recorded spike trains by our in-house software NPwaveX computing hundreds of parameters to describe the phenotypic effects in four well-established categories (general activity, synchronicity, burst structure, regularity). The established fingerprints reveal toxin- and MOA-specific parameter panels identified by multivariate classification analysis. We compare the results between human iPSC-derived neuronal networks and those from functionally mature primary mouse cortical networks.
In summary, our data show that the MEA technology allows dissecting the functional differences between hiPSC- and mouse neuronal culture model when treated with known neurotoxins and therefore provides a tool for investigating and comparing the safety margin of novel drug

candidates between rodent and human cell background and thereby complements the prediction of functional-neurotoxic and seizurogenic risk assessment.

Disclosures: **K. Jügelt:** A. Employment/Salary (full or part-time): NeuroProof GmbH. **A. Steder:** A. Employment/Salary (full or part-time): NeuroProof GmbH. **O.H.U. Schroeder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH. **B.M. Bader:** A. Employment/Salary (full or part-time): NeuroProof GmbH.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.19/EE12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CounterACT Program, National Institutes of Health, Office of the Director and the National Institute of Neurologic Disorders and Stroke [Grant Number 5U01NS058162-09]

Title: A rat model of nerve agent exposure applicable to the pediatric population: The anticonvulsant efficacies of atropine and GluK1 antagonists

Authors: *J. P. APLAND[1], S. L. MILLER[2], V. ARONIADOU-ANDERJASKA[3], T. H. FIGUEIREDO[2], E. M. PRAGER[4], C. P. ALMEIDA-SUHETT[4], M. F. M. BRAGA[3]; [1]Neurobehavioral Toxicol, USAMRICD, Gunpowder, MD; [2]Dept. of Psychiatry, [3]Dept. of Anatomy, Physiology, and Genetics, and Dept. of Psychiatry, [4]Dept. of Anatomy, Physiology, and Genet., USUHS, Bethesda, MD

Abstract: Inhibition of acetylcholinesterase (AChE) after nerve agent exposure induces status epilepticus (SE), which causes brain damage or death. The development of countermeasures appropriate for the pediatric population requires testing of anticonvulsant treatments in immature animals. In the present study, exposure of 21-day-old (P21) rats to different doses of soman, followed by probit analysis, produced an LD50 of 62 μg/kg. The onset of behaviorally observed SE was accompanied by a dramatic decrease in brain AChE activity; rats who did not develop SE had significantly less reduction of AChE activity in the basolateral amygdala than did rats who developed SE. Atropine sulfate (ATS) at 2 mg/kg, administered 20 min after soman exposure (1.2×LD50), terminated seizures. ATS at 0.5 mg/kg, given along with an oxime within 1 min after exposure, allowed testing of anticonvulsants at delayed time-points. The AMPA/GluK1 receptor antagonist LY293558 or the specific GluK1 antagonist UBP302, administered 1 h post-exposure, terminated SE. There were no degenerating neurons in soman-

exposed P21 rats, but both the amygdala and the hippocampus were smaller than in control rats at 30 and 90 days post-exposure; this pathology was not present in rats treated with LY293558. Behavioral deficits present at 30 days post-exposure were also prevented by LY293558 treatment. Thus, in immature animals, a single injection of atropine is sufficient to halt nerve agent-induced seizures, if administered timely. Testing anticonvulsants at delayed time-points requires early administration of ATS at a low dose, sufficient to counteract only peripheral toxicity. LY293558, administered 1 h post-exposure, prevents brain pathology and behavioral deficits.

Disclosures: **J.P. Apland:** None. **S.L. Miller:** None. **V. Aroniadou-Anderjaska:** None. **T.H. Figueiredo:** None. **E.M. Prager:** None. **C.P. Almeida-Suhett:** None. **M.F.M. Braga:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.20/EE13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA Grant 2.H_856_08_RC_C

Title: Pupillary light response in guinea pigs and swine exposed to organophosphate agents

Authors: E. D. CLARKSON[1], *M. C. MOFFETT[1], J. E. MORGAN[1], K. H. SMITH[1], S. M. SCHULZ[1], J. K. CHANDLER[1], C. L. ROUSAYNE[1], C. KOLANKO[2];
[1]USAMRICD, Aberdeen Proving Ground, MD; [2]Eyemarker Systems, Inc., Morgantown, WV

Abstract: Here we report a study to define and quantify the relationship between organophosphate agent exposure with acetylcholinesterase (AChE) inhibition and the ocular biomarkers that are induced. A guinea pig model was exposed to varying concentrations of parathion (pesticide), soman, and VX (nerve agents) to methodically detail both the temporal and quantitative occurrence of pupillary deficits (anticholinesterase biomarkers) to determine the most sensitive, accurate diagnostic algorithms in these animal models. Dose-response curves and temporal-response curves for both pupillary deficits and generalized symptoms were developed for each agent used. Based upon previous studies, dose ranges were chosen to incorporate lethal and sub-lethal exposures without decreasing the potential sensitivity of the ocular biomarkers. In addition, AChE assays were performed at various time points post-exposure over 24 hours. The key finding of this study was that, after exposure to organophosphates, changes in pupillary response were detected prior to changes in AChE levels in guinea pigs. We have conducted limited testing in swine in which VX at 1.7 LD_{50} (n=3) or 0.7 LD_{50} (n=3) was placed on a

swine's neck, and the animal was monitored over 6 hours. For the higher-dose animals, measurable AChE inhibition and pupillary response occurred at 1 hour and for the lower dose at 3 hours. The percent change in pupillary response was greater than the percent AChE inhibition. This opens the possibility of early detection of organophosphate exposure in a non-invasive manner that does not require the use of enzyme markers.
----[The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by the Defense Threat Reduction Agency - Joint Science and Technology Office, Medical S&T Division.]

Disclosures: **E.D. Clarkson:** None. **M.C. Moffett:** None. **J.E. Morgan:** None. **K.H. Smith:** None. **S.M. Schulz:** None. **J.K. Chandler:** None. **C.L. Rousayne:** None. **C. Kolanko:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

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Program#/Poster#: 423.21/EE14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: TUBITAK (The Scientific and Technological Research Council of Turkey) Grant 115S066

Title: Investigation of the resistance to glutamate-induced excitoxicity in mouse motor neuron-like NSC-34 cells on graphene oxide films

Authors: ***G. SENGUL**[1], S. TASDEMIR[2], P. CORUK[3], B. KAYHAN[3], A. SENDEMIR URKMEZ[4];
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Abstract: Excess glutamate release leads to glutamate-induced excitotoxicity and neuronal death by overstimulating NMDA-type glutamate receptors. Graphene, a monolayer of sp2-bonded carbon atoms, is a quasi-two-dimensional (2D) material with unique electrical and chemical properties, enabling electrical activity of the cells and facilitating the integration with neural

tissues. The aim of this *in vitro* study was to investigate the behavior of motor neurons on graphene oxide (an oxidized form of graphene) substrates and under glutamate-induced excitotoxicity which has been shown to be an important factor in many neurodegenerative diseases. For this purpose, we used graphene oxide film as a substrate for motor neuron-like NSC34 cells, a hybrid cell line produced by fusion of mouse neuroblastoma with mouse motor neuron-enriched primary spinal cord cells. NSC-34 cells were grown in DMEM high glucose formulation containing 10% fetal bovine serum. Cells were seeded on GO films and cells cultivated on tissue culture polystyrene were used as control. DMEM-F12 with reduced serum concentration (1%) and 1% non-essential amino acids as used for differentiation. After 6 days of differentiation, L-glutamic acid induced excitotoxicity was applied on NSC-34 cells on both surfaces. Following stress, morphologies of cultured neurons were examined by scanning electron microscopy (SEM) and immunostaining. Cell viability was measured by MTT assay. SEM results showed that the cells were attached on GO films. Cell viability (MTT) and toxicity (LDH) assays suggested a resistance to glutamate-induced excitoxicity in NSC-34 cells on graphene oxide films.

Disclosures: **G. Sengul:** None. **S. Tasdemir:** None. **P. Coruk:** None. **B. Kayhan:** None. **A. Sendemir Urkmez:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.22/EE15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibition of necroptosis by treatment with necrostatin-1 or RIP1 siRNA potentiates taxol-induced neuronal death via the activation of ERKs in mouse cortical cultures

Authors: *J.-K. KIM, S. HWANG;
Chonnam Nat'l Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract: Taxol is a well-known microtubule-stabilizing anticancer drug. We have previously reported that taxol induces oxidative neuronal apoptosis by enhancing the activity of NADPH oxidase in cultured cortical cells. Present study was performed to investigate whether necroptosis and autophagic cell death pathways may participate in the taxol-induced neuronal death (TIND). We examined the effects of some selective inhibitors of apoptosis, necroptosis and autophagy on the TIND in mouse cortical cultures. Twenty-four hour exposure to 300 nM taxol induced 40 - 60% neuronal death. The TIND was significantly attenuated not only by pretreatment with anti-apoptotic agents such as Z-VAD-FMK and Z-IETD-FMK but also by pretreatment with

autophagy inhibitors such as 3-methyadenine and bafilomycin A1. Unexpectedly, pretreatment with necrostatin-1 (Nec-1, 100 μM), a selective necroptosis inhibitor, significantly potentiated the TIND. Furthermore suppression of receptor-interacting protein 1 kinase (RIP1) using siRNA also potentiated the TIND. On the other hand, the TIND was significantly attenuated by pretreatment with SP600125, a c-Jun N-terminal Kinase inhibitor but not by PD098059, a mitogen activated and extracellular regulated kinase kinase 1 inhibitor or SB203580, a p38 MAPK inhibitor. However, the enhanced neuronal death by Nec-1 or RIP1 suppression was reversed by pretreatment with PD98059 as well as SP600125, not by SB203580. These findings demonstrate that treatment with Nec-1 or suppression of RIP1 expression may enhance TIND through the activation of ERKs and suggest that RIP1 inhibition may potentiate neuronal death in mouse cortical cultures.

Disclosures: J. Kim: None. **S. Hwang:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.23/EE16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Science and Technology

National Cheng Kung University Hospital

National Cheng Kung University

Title: Sex differences in oxaliplatin induced neuropathic pain behaviors

Authors: *L.-H. CHEN[1], M.-R. SHEN[2];
[1]Dept. of Pharmacol., [2]Obstetrics and Gynecology, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Current evidence suggests females show greater sensitivity and responses than males to chemotherapeutic drugs, including cisplatin and paclitaxel. However, sex differences in pain responses to oxaliplatin treatment still remain unclear. The aim of this study was to investigate the sex differences in mouse model of oxaliplatin-induced neuropathic pain. Mice were intraperitoneally injected with 7-mg/kg oxaliplatin on four alternate days or two cycles of 3-mg/kg oxaliplatin for 5 consecutive days, followed by 5 days of rest. In the mouse behavioral tests, oxaliplatin-induced cold allodynia was more robust in female mice; while no differences were observed between the two genders in oxaliplatin induced mechanical allodynia. Both female and male mice exhibited less locomotor activity following oxaliplatin injections. Electron

micrographs of sciatic nerves revealed that oxaliplatin caused small myelinated and non-myelinated fibers damages in both gender, large myelinated fiber damages by oxaliplatin were observed only in females. Taken together, our results suggest that females showed greater pain sensitivity and exhibited severe axonal damage in large myelinated axons of sciatic nerves after oxaliplatin treatments. In future studies, sex differences in pain responses to chemotherapeutic agents should be considered.

Disclosures: **L. Chen:** None. **M. Shen:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.24/EE17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The neurocognitive effects of vanadium in young male rats

Authors: *M. F. DE BUTTE;
Psychology, Sociology, and social work, West Texas A&M Univ., Canyon, TX

Abstract: Environmental exposure to heavy metals is a public health hazard that has been linked to cognitive and neurological deficits. Exposure to heavy metals can occur through contaminated food, water, air, or in industrial work settings. Of increasing interest in recent years are the health risks and/or benefits of exposure to the transition metal vanadium. Previous research on vanadium has shown evidence of toxicity when animals and humans are exposed including respiratory and gastrointestinal problems. Paradoxically, administration of vanadium salts has been found to ameliorate glucose tolerance as well as lower blood glucose levels in diabetic rodents as well as human patients. To date, the cognitive consequences of vanadium exposure are not well elucidated. Most studies on the effects of vanadium exposure on neurocognition examined its effects following moderate to large exposure. Hence, the purpose of the current study was to investigate whether chronic ingestion of a low dose of vanadium (.05mg/1000mg of food mash) would affect Open Field, Object Recognition, and Morris Water Maze performance in young male rats. Rats did not exhibit any signs of toxicity following the chronic ingestion of vanadium. Intriguingly, vanadium exposure improved spatial memory on day 2 of the Morris Water maze task. All rats exhibited normal locomotion and exploration when tested in the Open Field task. Vanadium exposure did not affect visual memory. This study indicates vanadium may have a positive impact on spatial memory warranting further research to better understand the potential benefits and consequences of this transition metal.

Disclosures: **M.F. De Butte:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

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Program#/Poster#: 423.25/EE18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: INSERM France

CNRST Maroc

NeuroMed travel grant

GDRI travel grant

Title: Impact of aflatoxin B1 on hypothalamic neuropeptides regulating feeding behavior

Authors: ***F. TREBAK**[1,2], A. ALAOUI[2], D. ALEXANDRE[1], S. ELOUEZZANI[2], Y. ANOUAR[1], N. CHARTREL[1], R. MAGOUL[2];
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Abstract: The presence of mycotoxins in food is a major problem of public health as they produce immunosuppressive, hepatotoxic and neurotoxic effects. Mycotoxins also induce mutagenic and carcinogenic effects after long exposure. Among mycotoxins that contaminate food are aflatoxins (AF) such as AFB1, which is the most powerful natural carcinogen. The AF poisoning results in symptoms of depression, anorexia, diarrhea, jaundice or anemia that can lead to death, but very few studies have explored the impact of AF on neuroendocrine regulations. To better understand the neurotoxic effects of AF related to anorexia, we explored in rat the impact of AFB1 on the major hypothalamic neuropeptides regulating feeding behavior, either orexigenic (NPY, Orexin, AgRP, MCH) or anorexigenic (α-MSH, CART, TRH). We also studied the effect of AFB1 on a novel neuropeptide, the secretogranin II (SgII)-derived peptide EM66, which has recently been linked to the control of food intake. For this, adult male rats were orally treated twice a week for 5 weeks with a low dose (150µg/kg) or a high dose (300µg/kg) of AFB1 dissolved in corn oil. Repeated exposure to AFB1 resulted in reduced body weight gain, which was highly significant for the high dose of AF. Immunocytochemical and quantitative PCR experiments revealed a dose-related decrease in the expression of all the hypothalamic neuropeptides studied in response to AFB1. Such orexigenic and anorexigenic alterations may

underlie appetite disorders as they are correlated to a dose-dependent decrease in body weight gain of treated rats as compared to controls. We also found a decrease in the number of EM66-containing neurons in the arcuate nucleus of AFB1-treated animals, which was associated with a lower expression of its precursor SgII. These findings show for the first time that repeated consumption of AFB1 disrupts the hypothalamic regulation of neuropeptides involved in feeding behavior, which may contribute to the lower body weight gain associated to AF exposure.

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Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.26/FF1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Parkin dependent mitophagy rescues retinal ganglion cell from ethambutol induced apoptosis

Authors: ***B. LEE**[1], H. JUN[2], J. KIM[2], J. KIM[1];
[1]Dept. of Biomed. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Ophthalmology, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: Ethambutol, one of the major component of combination pharmacotherapy regimen for drug resistance tuberculosis, is the most common causative agent of toxic optic neuropathy. Ethambutol have selective toxicity to retinal ganglion cell, but the mechanism of retinal ganglion cell death is obscure and there is no appropriate therapeutic measure for ethambutol induced toxic optic neuropathy. We found that ethambutol simultaneously induces caspase dependent apoptotic cell death and increment of autophagy flux in the retinal neuron both *in vivo* and *in vitro*. Ethambutol treatment resulted in a depolarization of mitochondrial membrane potential ($\Delta\Psi m$), transcriptional up-regulation of PINK1, PARK2, nuclear translocation of parkin, and phosphorylation of parkin at S65 in retinal neuronal cell. Ethambutol induced depolarization of $\Delta\Psi m$ and subsequent apoptosis of retinal neuron is exaggerated by si-RNA mediated *PARK2* knock-down. Co-treatment with rapamycin, an autophagy inducer, ameliorates the ethambutol induced depolarization of $\Delta\Psi m$ and caspase dependent apoptosis in retinal neuron, *in vitro.* Activation of mitophagy was evident in the retinal tissue of ethambutol induced toxic optic neuropathy mouse model and intravitreally delivered rapamycin significantly alleviated apoptosis of retinal ganglion cell. Collectively, mitophagy is a key neuroprotective mechanism in

ethmabutol induced toxic optic neuropathy and rapamycin facilitates this process in retinal neuron to protect retinal neuron from EMB induced apoptosis.

Disclosures: **B. Lee:** None. **H. Jun:** None. **J. Kim:** None. **J. Kim:** None.

Poster

423. Neurotoxic Agents

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.27/FF2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH T32 ES07051

Title: Additive deficits on egocentric and allocentric learning induced after developmental manganese overexposure combined with 6-hydroxydopamine striatal lesions

Authors: ***R. A. BAILEY**[1,2], A. GUTIERREZ[1,2], J. R. HUFGARD[1], T. L. KYSER[3,2], A. M. HEMMERLE[3,2], K. B. SEROOGY[3,2], C. V. VORHEES[1,2], M. T. WILLIAMS[1,2];
[1]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Neurosci. Grad. Program, Univ. of Cincinnati, Cincinnati, OH; [3]Neurol., Univ. of Cincinnati Col. of Med., Cincinnati, OH

Abstract: With neurodegenerative disorders on the rise, it has been hypothesized that environmental risk factors may play some role. Extreme manganese (Mn) overexposure (MnOE) can cause manganism, a disorder similar to, yet distinct from, Parkinson's disease (PD). Less extreme MnOE from air and water pollution is known to cause subtler effects, including being correlated with increased risk for PD. During early development, MnOE also poses risks, but whether this type of exposure predisposes individuals to Parkinsonism is unclear. To investigate this relationship, we gavaged Sprague-Dawley rats with MnCl (100 mg/kg, as a free metal) or NaCl vehicle from postnatal day (P)4 to P28 every other day. This dosing paradigm elevates blood Mn to levels similar to human exposure in areas where Mn pollution is prevalent. As adults, the animals were injected with 6-hydroxydopamine (6-OHDA) or 2% ascorbic acid into the neostriatum at a dose that does not induce motor effects. After recovery, the animals were assessed in an open-field and for egocentric learning in the Cincinnati water maze (CWM), allocentric learning in Morris water maze (MWM), and fear conditioning. After testing, the substantia nigra was examined for TH expression. Animals with MnOE or 6-OHDA alone showed deficits in egocentric and allocentric learning. These effects were increased in the group that received both exposures. MnOE had a larger effect on allocentric learning than 6-OHDA, while the effect of MnOE or 6-OHDA on egocentric learning was similar. In the open-field, MnOE animals had decreased mobility while 6-OHDA animals showed increased mobility.

Conditioned fear was not affected by MnOE or 6-OHDA. From initial cell counts, TH expression in the substantia nigra is lower in all experimental groups compared with the saline/sham controls. In conclusion, developmental MnOE may be a risk factor for Parkinsonism later in life. (Supported by NIH T32 ES07051).

Disclosures: **R.A. Bailey:** None. **A. Gutierrez:** None. **J.R. Hufgard:** None. **T.L. Kyser:** None. **A.M. Hemmerle:** None. **K.B. Seroogy:** None. **C.V. Vorhees:** None. **M.T. Williams:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1GM112831

Title: Effects of testosterone on cell death and cognitive deficits following postnatal exposure to isoflurane

Authors: *J. SASAKI RUSSELL, J. LEONG, M. ROTMAN, J. CHAN, J. SALL;
Anesthesia and Perioperative Care, Univ. of California, San Francisco, San Francisco, CA

Abstract: Exposure to general anesthesia during early development induces neuronal death and cognitive deficits later in life in animal models. Recent studies suggest that males are more negatively impacted by anesthesia than females, however the mechanism underlying this difference is unknown. To investigate whether the presence of testosterone in the postnatal brain during anesthesia exposure leads to or aggravates the resulting cognitive deficits, male Sprague Dawley rats underwent gonadectomy at postnatal day 2 (P2) followed by exposure to 6 hours of clinically relevant concentrations of volatile anesthetic isoflurane at P7. Non-gonadectomized littermate control groups were simultaneously exposed to either isoflurane or room air. All pups were separated from dams for the same length of time. Rats were weaned at P21 and subjected to a series of object recognition and association tasks beginning at P37. All groups performed similarly well on the Novel Object Recognition task, however the isoflurane-alone group exhibited a decreased performance in some of the more complex object recognition tasks, as seen in previous studies. This deficit was ameliorated in the isoflurane-exposed gonadectomized group. Next, we examined whether the extent of isoflurane-induced cell death that is consistently observed immediately after exposure was altered in gonadectomized rats. In a separate cohort of gonadectomized and control rats, cell death in the thalamus and hippocampus was assessed 12

hours following the anesthetic or air exposure. Cell death was similar between both isoflurane-exposed groups, regardless of gonadectomy, and was significantly higher in both groups when compared to the air-exposed group. Our results indicate that the absence of testosterone does not block cell death after anesthesia in specific brain regions of interest, however does provide some level of neuroprotection, as evidenced by the cognitive tests during adulthood. These findings suggest that the presence of testosterone during anesthetic exposure may be mechanistically involved in anesthesia-induced injury in the developing brain on a synaptic or circuit level.

Disclosures: **J. Sasaki Russell:** None. **J. Leong:** None. **M. Rotman:** None. **J. Chan:** None. **J. Sall:** None.

Poster

423. Neurotoxic Agents

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 423.29/FF4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Resting State fMRI of Gulf War Illness patients reveals abnormal connectivity within and between different brain function networks consistent with the multi-symptom illness

Authors: *K. GOPINATH[1], V. KRISHNAMURTHY[1], L. KRISHNAMURTHY[2], B. THAPA-CHETRY[2], L. OUYANG[2], A. GOYAL[2], P. GANDHI[2], Y. FANG[2], R. BRIGGS[3], R. HALEY[2]; [1]Radiology & Imaging Sci., Emory Univ., Atlanta, GA; [2]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [3]Univ. of Florida, Gainesville, FL

Abstract: Up to 250,000 veterans of the 1991 Gulf War suffer from illness (GWI) characterized by multiple deficits in cognitive, emotion, somatosensory and pain domains. In this study we employed resting state fMRI (rsFMRI) to map impairments in brain function in GWI with advanced network analysis. 22 veterans with GWI (mean age 49.4 yrs.) and 30 normal controls (NC) (mean age 49.8 yrs.), were scanned in a Siemens 3T MRI scanner using a 12-channel Rx head coil. Written informed consent was obtained from all participants in the protocol approved by the local Institutional Review Board. rsFMRI data were acquired with a 10-min whole-brain gradient echo EPI (TR/TE/FA = 2000/24ms/90°, resolution = 3mm x 3mm x 3.5mm). The preprocessed rsFMRI data for each subject was parcellated using the AAL ROI atlas to construct a 116 node graph. The distance matrix of the graph was formed by the z-transformed cross-correlation coefficients between all nodes' ROI-averaged grey matter voxel time-series. Network based statistics (NBS) was then employed to yield significant (5000 permutations based multiple comparisons corrected $p < 0.05$) connected networks of edges which exhibit abnormally decreased or increased rsFC in GWI patients. NBS yielded one *significant* connected network

comprised of 114 AAL nodes and 404 edges which exhibited abnormally increased rsFC in GWI compared to NC (GWS > NC); and another *significant* network with 102 nodes and 228 edges which exhibited decreased rsFC in GWI (GWS < NC). The large extant of these networks indicate brain-wide impairments consistent with the multi-symptom nature of GWI. In order to further probe the brain regions which exhibited most impaired/abnormal rsFC in GWI within these two networks, binary distance matrix sub-graphs were formed for each NBS network (GWS > NC and GWS < NC) by thresholding the corresponding t-statistic matrix at 9 different p-values in the range 0.025-0.0005. The mean of the 9 degree centrality (DC) datasets was employed to assess the nodes with most impaired/abnormal rsFC in GWI. Examination of the nodes with highest DC and their associated networks revealed that GWI patients exhibit significantly ***reduced rsFC*** in sensorimotor networks and basal ganglia thalamocortical networks involved in cognition and regulation of emotion, consistent with deficits in cognitive and sensory domains seen in GWI. On the other hand GWI patients exhibit abnormally ***increased rsFC*** between somatosensory network and areas in limbic, salience and pain processing networks consistent with central pain and sensory hypersensitivity seen in GWI. Thus a strong correspondence is observed between rsFC network impairments and brain function deficits in GWI.

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Poster

423. Neurotoxic Agents

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Program#/Poster#: 423.30/FF5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Faculty Research Grant

Murphy Fellowship

Fletcher Jones Fellowship

Roberts Fellowship

Title: Neurodevelopmental effects of organophosphate pesticide exposure

Authors: *E. A. FRADINGER, B. AHN, G. X. GARCIA, H. R. SCHMIDT, H. GARCIA, O. MAC, D. B. BOURGAIZE;
Biol. Dept., Whittier Col., Whittier, CA

Abstract: Organophosphate pesticides irreversibly and semi-permanently inhibit acetylcholinesterase causing an accumulation of acetylcholine at the synapse and hyperstimulation of the cholinergic neurons in the CNS and PNS. This study seeks to understand the acute effects of organophosphate pesticide exposure on developing neurons both *in vivo* and *in vitro*. Using the zebrafish model system we have shown that developmental organophosphate exposure inhibits acetylcholinesterase activity and causes changes to acetylcholine-dependent physiological processes including an increase in spontaneous movement generation at 24 hours post-fertilization and bradycardia at 48 hours post-fertilization. To further investigate the effects of these pesticides on neuronal development we have utilized the cholinergic PC-12 cell line. Differentiating PC-12 cells were shown to be sensitive to organophosphate exposure at mid to late stages of differentiation when the neurons were beginning to form synapses. At earlier stages, pesticide exposure had little effect on cell survival. We then examined the effect of organophosphate exposure on neuronal morphology and architecture. Interestingly, the toxicity of the organophosphate pesticide diazinon appears to be independent of acetylcholinesterase inhibition. Together these data demonstrate that organophosphate exposure adversely affects neuronal development and highlights the importance of understanding the neurodevelopmental effects of USDA approved pesticides.

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Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R37 NS041435

Title: Noggin inhibits bmp signaling in oligodendrocyte progenitor cells to repress transdifferentiation into astrocytes.

Authors: *H. STRASBURGER[1], L. KIRBY[2], J. SCHOTT[2];
[1]Johns Hopkins Hosp., Baltimore, MD; [2]Johns Hopkins Neuroimmunology, Baltimore, MD

Abstract: Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system involving inflammation and neurodegeneration. In MS, the oligodendrocytes die off and axons are demyelinated. One possible therapeutic for MS is to induce more efficient remyelination of axons by targeting endogenous oligodendrocyte progenitor cells (OPCs) to replenish mature oligodendrocytes lost as a result of disease. In order to induce endogenous remyelination, the signaling factors surrounding oligodendrocyte differentiation and maturation must be investigated. We hypothesized that within an inflammatory environment, some OPCs may leave the oligodendrocyte lineage and instead transdifferentiate to become astrocytes. It has been observed in MS patients that there is a reduced production of noggin, a BMP inhibitor, and disregulated levels of BMPs. BMP binds to receptors BMPR1 and BMPR2 leading to Smad signaling within the cell. SMAD 1/5/8/ is phosphorylated once the BMPRs dimerize and binds to Smad4 to translocate to the nucleus to act as a transcription factor. If BMP signaling leads to increased glial fibrillary acidic protein (GFAP), an astrocyte specific protein, OPCs could potentially leave the oligodendrocyte lineage and transdifferentiate into astrocytes. Increased levels of astrocytes could further inflammation and neurodegeneration. Our results show that in rat OPC cultures the presence of noggin astrocyte contamination is greatly reduced in comparison to cultures without noggin. Using PDGFαR-CreER;Rosa26-eYFP mice we isolated OPCs at P5 and tracked OPC Cell fate in vitro with and without noggin. It was found that in the presence of noggin, transdifferentation of OPCs into astrocytes was inhibited, whereas without noggin 15% of the OPCs had left the oligodendrocyte lineage to become astrocytes as determined by immunocytochemistry. The same PDGFαR-CreER;Rosa26-eYFP mice were used to determine if transdifferentiation occurs in vivo. Mice were fed cuprizone for 6 weeks and a cocktail of BMP4/5 was administered through an osmotic pump into the brain. It was observed that at areas near the cannula insertion into the cortex OPCs had transdifferentiated into astrocytes. In addition, significant gliosis was observed near the injection site. These results suggest that BMP plays a role in determining OPC fate and gliosis. We are beginning to elucidate the Noggin-BMP balance in vivo to determine its affect on both astrocytes and OPC differentiation.

Disclosures: **H. Strasburger:** None. **L. Kirby:** None. **J. Schott:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: ANPCyT - PICT 1563

CONICET - PIP 0446

Title: Neuroprotective effect of melatonin in experimental optic neuritis in rats

Authors: *M. L. ARANDA, M. F. GONZALEZ FLEITAS, M. I. KELLER SARMIENTO, M. S. CHIANELLI, P. H. SANDE, D. DORFMAN, R. E. ROSENSTEIN;
CEFyBO - CONICET, Buenos Aires, Argentina

Abstract: We have developed a new experimental model of optic neuritis (ON) through the microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve, which reproduces central features of human ON. The aim of this work was to analyze the effect of melatonin (MEL) on the optic nerve axoglial alterations induced by experimental ON. For this purpose, LPS (1 μl, 4.5 μg) was injected in one optic nerve from adult male Wistar rats, while the contralateral optic nerve was injected with vehicle. One group of animals received a subcutaneous pellet of MEL (20 mg) one day before LPS or vehicle injection which was replaced at 15 days, and another group was submitted to a sham procedure. In another set of experiments, the pellet of melatonin was implanted at 4 days post-injection of LPS or vehicle. The effect of melatonin was analyzed at 21 days post-injection in terms of: i) visual pathway function (visual evoked potentials (VEPs)), ii) anterograde transport from the retina to the superior colliculus (intravitreal injection of cholera toxin β-subunit), iii) pupil light reflex (PLR), iv) microglia/macrophages (by Iba-1 and ED1 immunoreactivity), v) astrocytes (by glial fibrillary acid protein-immunostaining), vi) axon number (by toluidine blue staining), vii) demyelination (by luxol fast blue staining), viii) retinal ganglion cells (RGCs) number (by Brn3a immunoreactivity), and iv) optic nerve lipid peroxidation (TBARS). LPS induced a significant decrease in VEP amplitude and PLR, a reduction in retinal anterograde transport, an increase in Iba-1 and ED1 immunoreactivity, astrocytosis, demyelization, an increase in lipid peroxidation, and RGC loss. The pre-treatment with MEL significantly prevented all these alterations. The post-treatment with MEL significantly preserved VEP amplitude and PLR. The treatment with melatonin prevented functional and histological alterations and diminished the vulnerability of RGC to the deleterious effects of experimental ON, probably through an antioxidant mechanism. Therefore, these results indicate that melatonin could be a promissory resource in the management of ON.

Disclosures: **M.L. Aranda:** None. **M.F. Gonzalez Fleitas:** None. **M.I. Keller Sarmiento:** None. **M.S. Chianelli:** None. **P.H. Sande:** None. **D. Dorfman:** None. **R.E. Rosenstein:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.03/FF8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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NIHR Mental Health Biomedical Research Centre at South London and Maudsley NHS Foundation Trust

Title: Serum factors as predictors of interferon-alpha (IFN-alpha)-induced depression

Authors: *A. BORSINI, P. ZUNSZAIN, C. PARIANTE, S. THURET;
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Abstract: IFN-α is the standard treatment for hepatitis C virus (HCV) infection, which causes high rates of depression. However, the biological factors that predispose an individual to the occurrence of depression are still unknown. Previous data have reported an alteration in the serum level of inflammatory markers in depressed, when compared with non-depressed patients. There is in fact evidence for serum factors to penetrate the blood brain barrier and modulate different brain signalling. Our study proposes to investigate whether co-incubation of human hippocampal progenitor cells (HPCs) with serum from IFN-α-treated HCV patients differently affect cell biology, when comparing serum from patients who will and will not later develop depression. Serum samples were collected at baseline, before the IFN-α treatment begins (treatment week (TW) 0) and at TW4 from 33 HCV patients; 9 of these patients later developed IFN-α-induced depression. The multipotent human hippocampal progenitor cell line HPC03A/07 was used to evaluate the effects of serum. Cells were co-incubated with serum samples under proliferating conditions for 2 days, followed by differentiating conditions for 7 days. During proliferation, apoptotic cells were evaluated by immunostaining with caspase 3 (CC3), whereas neuronal differentiation was assessed with doublecortin (DCX). Treatment with TW0 serum from depressed patients increased the percentage of CC3+cells ($U=49.5$, $p<0.05$), when compared with serum from non-depressed. However, there was no difference in the percentage of DCX+cells ($U=81$, $p=0.3$) between the two groups. In contrast, treatment with TW4 serum from non-depressed patients increased the percentage of DCX+cells ($U=56$, $p<0.05$), when compared with depressed. However, there was no significant difference in the percentage of

CC3+cells (U=100, p=0.8) between the two groups. Indeed, the increase in the percentage of DCX+cells was significantly higher upon treatment with serum samples from non-depressed than from depressed patients, when comparing TW4 with TW0 ($F(1,31)=6.5$; $p<0.05$). No significant difference was reported in the percentage of CC3+cells between depressed and non-depressed patients, when comparing TW4 with TW0 ($F(1,31)=0.8$; $p=0.4$). Our findings show that blood factors contained in serum of HCV patients who will later develop depression modulate both the process of apoptosis and neuronal differentiation. Future analyses should allow for the detection of serum factors involved in the alteration of cell death and neurogenesis, which may contribute to the advancement of novel therapeutic strategies for the prevention of IFN-α-induced depression.

Disclosures: **A. Borsini:** None. **P. Zunszain:** None. **C. Pariante:** None. **S. Thuret:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.04/FF9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FP7 Marie Curie nEUROinflammation

Title: Pivotal role of the macrophage colony stimulating factor (CSF1) in experimental allergic encephalomyelitis

Authors: ***N. BORJINI**[1,2,5], M. FERNANDEZ[2], L. GIARDINO[2,5,3], L. CALZÀ[2,5,4];
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Abstract: Inflammation is a common neuropathological feature in multiple sclerosis (MS) and EAE (experimental allergic encephalomyelitis), the most widely used animal model of MS. However, very early molecular signature of neuroinflammation in EAE is still obscure, such as the potential link between tissue alterations and peripheral biomarkers. In order to elucidate these points, in the present study we investigated molecular mediators of neuroinflammation and demyelination in the tissue (SC), CSF and plasma in female Dark-Agouti rats during early presymptomatic EAE, using high-throughput technologies for gene expression and protein assays. At 1, 5, 8, 11 and 18 day post-immunization (DPI), EAE rats were sacrificed and samples were collected. Our results indicate that the profile of neuroinflammation and demyelination

biomarkers is dramatically changed during the early phase of EAE in tissue, CSF and plasma. In particular, we have identified the regulation of the chemokine colony stimulating factor 1 (CSF1) at 1 DPI in EAE groups compared with control, that was decreased in tissue (mRNA, P=0.0064), decreased in CSF and increased in plasma (protein, P=0.0006). It was then attempted to identify the cell type producing CSF1 in the CNS by double labeling immunohistochemistry experiments approach, and we found that at 1 DPI CSF1 is expressed by oligodendrocytes and astrocytes in EAE animals, while at 11DPI (acute phase) only astrocytes express CSF1. Overall our findings suggest an early role of CSF1 in the course of MS pathology, and a potential, new target for MS.

Disclosures: **N. Borjini:** None. **M. Fernandez:** None. **L. Giardino:** None. **L. Calzà:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.05/FF10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: UK MS Society 978/12

Title: Persistent cytokine production induced in the cerebral meninges in a rat model of MS gives rise to chronic cortical pathology

Authors: ***R. REYNOLDS**, R. JAMES, E. BROWNE, L. FUENTES, N. MAZARAKIS; Imperial Col. London, London, United Kingdom

Abstract: The progressive phase of multiple sclerosis (MS) is characterised by accumulating grey matter (GM) pathology, including demyelination, neuronal and axonal damage and loss. Cortical demyelination is closely associated with the presence of immune cell infiltrates in the meninges, including lymphoid-like tissue development. Analysis of isolated meninges of secondary progressive MS cases has shown increased gene expression for the pro-inflammatory cytokines: tumour necrosis factor (TNF), lymphotoxin-α (LTα) and interferon-γ (IFNγ); and for the chemokines CXCL9 and CXCL13. In order to test the hypothesis that chronic production of pro-inflammatory cytokines in the meningeal compartment and diffusion into underlying GM can drive MS GM pathology, we stereotactically injected HIV-1 based VSV-g pseudotyped lentiviral transfer vectors into the sagittal sulcus (SS) of DA rats to deliver continuous transgene expression (TNF + IFNγ or LTα + IFNγ) in the meninges in a chronic manner. A neuropathology analysis was conducted at time points up to 3 months, together with RT-PCR to determine changes to TNF receptor-1 (TNFR1) signalling. Efficient transduction of meningeal cells resulted in cytokine expression for up to 3 months. Injection of vectors for TNF or LTα, in

combination with IFNγ, induced the formation of large immune cell aggregates in the meninges by 28 dpi, which remained at 3 months, containing CD4+ and CD8+ T-cells, CD79a+ B-cells and Iba1+ macrophages. These aggregates extended the length of the SS and across the surface of the cortex. Subpial demyelination was accompanied by widespread microglial activation underlying these cellular aggregates. Demyelination was increased in rats pre-immunised with a low dose of myelin protein MOG. A decrease in neurofilament expression in regions with subpial demyelination was present along with signs of neuronal stress indicated by Fluorojade-C staining of layer II-IV neurons. TNF/TNFR1 interaction can initiate cell death by activating pathways involved in necroptosis. TNF and IFNγ vector injected animals at 28 dpi showed an increase in expression of TNFR1 and downstream necroptotic genes and proteins, RIP3 and MLKL and their phosphorylated derivatives, compared to eGFP vector control animals. RIP3+ and MLKL+ immunopositive cells with the morphology of neurons were present in TNF + IFNγ vector injected animals. Our results suggest that TNF and LTα in the presence of IFNγ are potent inducers of meningeal inflammation and can activate TNF signalling pathways in cortical cells leading to neuronal death and subpial demyelination and thus may contribute to clinical progression in MS.

Disclosures: R. Reynolds: None. **R. James:** None. **E. Browne:** None. **L. Fuentes:** None. **N. Mazarakis:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.06/FF11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Else-Kroener-Fresenius Foundation

Title: Beneficial effects of nimodipine in EAE

Authors: *A. SCHAMPEL[1], S. KUERTEN[2];
[1]Anat. and Cellbiology, [2]Anat. and Cell Biol., Univ. of Wuerzburg, Wuerzburg, Germany

Abstract: Common therapeutic strategies in multiple sclerosis (MS) research aim at attenuating the immune response but do not provide persistent prevention of neurodegeneration. To gain insight into the underlying pathomechanisms of neurodegeneration and axonal pathology experimental autoimmune encephalomyelitis (EAE) is widely used as animal model for MS. In our study EAE was induced in SJL/J mice by subcutaneous injection of MP4 in complete Freund´s adjuvant. Immunization with this myelin antigen leads to a relapsing-remitting form of

disease. After immunization mice were observed daily for clinical symptoms and rated according to the common EAE-scoring system. The L-type calcium channel antagonist nimodipine is known to induce beneficial effects on cognitive performance, aging and on local vascularization of the central nervous system. However the effect of nimodipine on inflammatory-mediated neurodegenerative diseases has not been well studied yet. This study is the first to examine the effect of long-term treatment with nimodipine in a relapsing-remitting form of EAE. Our data show that the course of EAE was significantly reduced in nimodipine-treated mice compared to vehicle-treated littermates. Attenuation of clinical symptoms was reflected by less nerve fiber pathology and enhanced remyelination in ultrastructural analyses of the spinal cord. We observed several ways how nimodipine could favor remyelination in EAE, organotypic spinal cord culture and cell culture. Those mechanisms are still investigated but our results already indicate that nimodipine might be an important tool for therapeutic strategies.

Disclosures: **A. Schampel:** None. **S. Kuerten:** None.

Poster

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Title: Altered functional Connectivity of striatal subregions in patients with multiple sclerosis

Authors: ***F. CUI**[1,2], L. ZHOU[2], K. JORGENSON[1], Z. WANG[1], Y. YU[2], Y. GAO[2], J. KONG[1]; [1]Massachusetts Gen. Hospital/Harvard Med. Sch., Charlestown, MA; [2]Dongzhimen Hosp., Beijing, China

Abstract: Introduction The striatum, a component of the basal ganglia, is involved in diverse functional domains including movement, cognition, emotion and reward. Recently, studies have shown that the striatum is also involved in the neuropathology of multiple sclerosis (MS). In this

study, we systemically compared the rsFC of striatal subregions between MS patients and matched healthy controls.
Methods Multiple sclerosis patients in the remission stage and healthy controls matched on age and gender were recruited for this study. Each subject participated in an identical fMRI scanning session using a 3T Siemens MRI system, in which 10-minute resting state fMRI data were collected. Seed-based rsFC was analyzed with CONN. We subdivided striatal subregions into the following 6 seed regions: dorsal caudate (DC), ventral caudate (superior) (VSs), ventral caudate/nucleus accumbens (inferior) (VSi), dorsal rostral putamen (DRP), dorsal caudal putamen (DCP), and ventral rostral putamen (VRP). A threshold of voxelwise $p< 0.005$ and $p< 0.05$ FDR corrected was applied.
Results Twenty MS patients and 15 healthy controls completed the study. The mean age of subjects was 36.97 ± 12.01 (mean ± SD). There were no significant differences in age and gender at baseline. The rsFC analysis showed that compared to the healthy controls, the MS patients produced significantly greater rsFC between 1) left DC and left precuneous, bilateral precentral, postcentral gyrus, and left parahippocampus; 2) left DCP and left cerebellum, middle frontal gyrus, and supramarginal gyrus; 3) left VSs and right lateral occipital cortex, middle temporal gyrus, and angular gyrus; 4) right VSs and left hippocampus, and parahippocampal gyrus. In addition, we found significant decreases in rsFC between 1) left DC and right cerebellum, left middle frontal gyrus (premotor), right parahippocampus; 2) right DC and right thalamus, insular, putamen, 3) right VRP and bilateral middle frontal gyrus (premotor), precentral gyrus, left superior frontal gyrus; 4) left VSs and left middle frontal gyrus (premotor), middle temporal gyrus; 5) right VSs and right middle prefrontal cortex.
Conclusion We identified a number of intriguing, statistically significant changes in resting state connectivity of the striatum subregions between MS patients and healthy controls. Our results suggest that different subregions of the striatum, especially dorsal caudate, putamen and ventral putamen, play important roles in movement regulation and modulation in MS patients.

Disclosures: **F. Cui:** None. **L. zhou:** None. **K. Jorgenson:** None. **Z. Wang:** None. **Y. yu:** None. **Y. gao:** None. **J. kong:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.08/FF13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effect of ONO-2952, a novel translocator protein 18 kDa antagonist, in a mouse model of multiple sclerosis

Authors: M. ISHISAKA, T. KOMIYA, T. KITAJIMA, *A. KISHI, S. KATSUMATA;
ONO Pharmaceut. Co., Ltd, Osaka, Japan

Abstract: Background: Translocator protein 18 kDa (TSPO) is localized in the outer mitochondrial membrane of many cell types such as reactive astrocyte and its expression is found to be up-regulated under various pathological conditions such as inflammation, mechanical lesions, neurological diseases, and cancer. In this study, we investigated the effect of ONO-2952 in the mouse model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE).
Methods: EAE was induced in 8-week-old female C57BL/6J mice by injecting an emulsion of 200 μg of Myelin Oligodendrocyte Glycoprotein (MOG35-55) peptide in Freund's Complete Adjuvant (FCA) containing 500 μg of heat-inactivated M. tuberculosis (H37RA) subcutaneously on flank (Day 0). In addition, 200 ng of pertussis toxin (PTX) was intravenously injected on the same day as MOG-FCA, and a second PTX injection was administered 48 h later (Day 2). ONO-2952 (10 and 30 mg/kg/day) or vehicle (0.5% methylcellulose) were orally administered once a day for 30 days from the day before MOG-FCA injection. Mice were monitored daily for clinical signs of EAE and scored as follows: 1, flaccid tail; 2, weakness of hind limbs; 3, paralysis of both hind limbs; 4, paralysis of all limbs; and 5, moribund. On Day 29, mice were anesthetized and blood and spinal cord were collected. Immunostaining was performed using L4-L5 spinal cord sections to investigate the activation of astrocyte and microglia cells.
Results: ONO-2952 30 mg/kg/day ameliorated the daily neurological score and significantly improved the accumulated neurological score compared with vehicle-treated group. On Day 29, the numbers of GFAP-positive astrocyte and Iba1-positive microglia were increased in the spinal cord and ONO-2952 significantly decreased the population of GFAP-positive astrocyte and Iba1-positive microglia in a dose-dependent manner. There were no significant changes in the numbers of peripheral lymphocytes and neutrophils in ONO-2952-treated group.
Conclusions: These observations suggest that the novel TSPO antagonist, ONO-2952, is protective in the mouse model of MS, and a possible mechanism involved in the effect of ONO-2952 is to inhibit glial cell activation. ONO-2952 could be a potential new therapeutic option for MS without affecting peripheral immune cells.

Disclosures: M. Ishisaka: A. Employment/Salary (full or part-time): ONO PHARMACEUTICAL CO., LTD. **T. Komiya:** A. Employment/Salary (full or part-time): ONO PHARMACEUTICAL CO., LTD. **T. Kitajima:** A. Employment/Salary (full or part-time): ONO PHARMACEUTICAL CO., LTD. **A. Kishi:** A. Employment/Salary (full or part-time): ONO PHARMACEUTICAL CO., LTD. **S. Katsumata:** A. Employment/Salary (full or part-time): ONO PHARMACEUTICAL CO., LTD..

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R37 NS041435

MedImmune

Title: Local administration of TH2 cells into the CNS ameliorates the inhibitory effects of IFNg on remyelination

Authors: *L. A. KIRBY, M. SMITH, J. SCHOTT, P. CALABRESI;
Neuroimmunology, Johns Hopkins, Baltimore, MD

Abstract: Multiple sclerosis (MS) is an immune mediated demyelinating disease of the CNS. One potential cause of remyelination failure is chronic inflammation, therefore understanding the molecular mechanisms by which CNS inflammation inhibits the oligodendrocyte precursor cells (OPC) is important. While effector T-cells of the TH1 and TH17 phenotype are understood to be critical for the pathogenesis of MS, effector T-cells of the TH2 phenotype may have an opposing role by promoting remyelination and repair especially in an inflammatory setting. To investigate the role of TH2 cells we utilized the GFAP/tTA; TRE/IFNg transgenic mouse line in which CNS expression of IFNg from GFAP positive astrocytes is achieved upon doxycycline withdrawal. In order to determine whether TH2 cells promote remyelination after cuprizone induced demyelination we first directly administered TH2 cells (glatiramer reactive cells) into the CNS via intraventricular microinjection. In three separate experiments remyelination was significantly improved in TH2 injected mice compared to PBS injected control animals (mean percent difference = 18.91; p-value = 0.0107) as determined by black gold staining in corpora callosa tissue. Interestingly, the TH2 effect of improved remyelination was only detected in cuprizone fed mice induced to express IFNg in the CNS and there was no significant difference between TH2 and control mice in which IFNg expression was suppressed. The fact that TH2 cells can reproducibly overcome the strong and potent inhibitory effect of IFNg and promote remyelination is notable. While it remains controversial whether peripheral administration of TH2 cells are able to cross the intact BBB and accumulate in the CNS, we have been unable to detect a CD4+ cell population 14 days after adoptive transfer into cuprizone fed GFAP/tTA; TRE/IFNg mice. In order to further probe the mechanisms by which TH2 cells are mediating their effect, we are examining a wide variety of cytokines and neurotrophic factors including IL-4 and BDNF, an in vitro OPC differentiation assay. While only modest effects on OPC differentiation were observed in the IFNg/IL-4 co-treatment paradigm as determined by MBP mRNA and protein expression. Future work will be directed at elucidating the potential role of

BDNF and other factors, both *in vitro* and *in vivo* using the GFAP/tTA; TRE/IFNg transgenic mice. Further work in identifying the mechanism could yield a novel therapeutic target for MS.

Disclosures: **L.A. Kirby:** None. **M. Smith:** None. **J. Schott:** None. **P. Calabresi:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Title: Astrocyte remodeling precedes extracellular matrix modifications in the glial lamina of mice with glaucomatous optic neuropathy

Authors: *R. A. FISCHER[1,2], H. L. MALLARO[3], E. S. BUYS[4,5,6,7], R. M. SAPPINGTON[1,3]; [1]Dept. of Pharmacol., [2]Pharmacol. Grad. Program, Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Eye Inst., Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Anesthesia Ctr. for Critical Care Res., [5]Dept. of Anesthesia, [6]Critical Care and Pain Med., [7]Dept. of Ophthalmology, Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Glaucoma is one of the leading causes of irreversible blindness in the world and results from degeneration of retinal ganglion cell (RGC) axons in the optic nerve. The two main risk factors are advanced age and elevated intraocular pressure (IOP). The optic nerve head (ONH) is considered the primary site of injury to RGC axons and contains the glia lamina that is comprised of astrocytes and demarcates the transition between unmyelinated and myelinated segments of RGC axons. ONH astrocytes provide structural support for RGC axons, in part by mediating extracellular matrix (ECM). Elevated IOP can alter the elasticity of the ONH and promote further progression of RGC degeneration. To determine how temporal changes in IOP alter astrocyte and ECM remodeling in glaucoma, we examined changes in the ECM as well as cytoarchitecture of astrocytes in the ONH and myelinated optic nerve (mON) of three mouse

models of glaucoma. To examine short-term IOP elevations, we utilized an inducible model (Microbead Occlusion Model). For long-term IOP elevations, we examined two chronic models, soluble guanylate cyclase α_1 deficient (sGCα_1-/-) and DBA/2J mice, in which IOP elevations are age-dependent. To measure changes in astrocyte cytoarchitecture, we labeled actin (phalloidin) and intermediate (GFAP) filaments and quantified changes in filament organization. In corresponding tissue sections, we performed immunolabeling and quantification of ECM components, including collagen I, collagen IV, TGF-β, and thrombospondin. Disorganization of actin and intermediate filaments in both ONH and mON astrocytes was apparent after short-term elevation of IOP (4 weeks) and persisted for long-term elevations (2-4 months). Actin labeling was significantly reduced in ONH astrocytes across all models ($p < 0.05$). In the ONH of all models, fiber orientation in astrocytes shifted from perpendicular to parallel, with respect to RGC axon bundles ($p < 0.05$). These changes in ONH astrocytes were accompanied by decreased collagen I and collagen IV in the ECM of DBA/2J and sGCα_1-/- mice, respectively ($p < 0.05$). In the mON, astrocyte reorganization coincided with decreased collagen IV and TGF-β in the ECM of sGCα_1-/- and DBA/2 mice, respectively ($p < 0.05$). Short-term elevations in IOP did not alter ONH ECM and only increased thrombospondin composition in the mON ($p < 0.05$). Our data suggest that elevated IOP induces relatively early and persistent astrocyte remodeling. Changes in ECM composition noted in chronic models could contribute to increased elasticity of the ONH and are likely preceded by astrocyte remodeling, as evidenced by the lack of ECM changes in the ONH following short-term IOP elevation.

Disclosures: R.A. Fischer: None. **H.L. Mallaro:** None. **E.S. Buys:** None. **R.M. Sappington:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.11/FF16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cuprizone treatment, toxic demyelination, and the blood-brain barrier

Authors: *J. SHELESTAK[1], R. CUKELJ[2], N. SINGHAL[1], J. MCDONOUGH[1], E. FREEMAN[1], R. CLEMENTS[1];
[1]Biomed. Sci., [2]Kent State Univ., Kent, OH

Abstract: The cuprizone animal model is widely used to study toxic demyelination in the central nervous system. Cuprizone is a copper chelator that causes selective oligodendrocyte death leading to consistent demyelination. Unlike most demyelinating diseases such as MS, existing

studies suggest the cuprizone model appears to maintain an intact blood-brain barrier (BBB). In the present study, we aim to assess the integrity of the blood brain barrier after 6 week administration of cuprizone. Mice were fed with cuprizone supplemented in the diet for 6 weeks and imaged using MRI every two weeks starting on week 0. Evidence of demyelination and ventricle enlargement was examined with T1, T2, and diffusion weighted protocols. The mice were sacrificed after 6 weeks of treatment and tissue sections were immunofluorescently stained and imaged using confocal microscopy. Tissue was stained for neurons, myelin, astrocytes, microvessels, oligodendrocytes and immune cells and analyzed to assess the integrity of the blood-brain barrier. Cuprizone treatment caused significant weight loss, as well as a measurable change in ventricle size seen after 6 weeks of treatment. Myelin staining intensity was clearly reduced in distinct brain regions including primary motor cortex, corpus callosum, and striatum. There was a significant increase in detectable astrocytes, as well as alterations in both astrocytic and microglial morphologies indicative of cellular activation. BBB integrity was assessed using computational image analysis techniques to investigate whether cuprizone treatment is associated with morphological changes indicative of BBB disruption. Here we report the status of BBB integrity and its relationship to demyelinating activity in the cuprizone animal model.

Disclosures: J. Shelestak: None. **R. Cukelj:** None. **N. Singhal:** None. **J. McDonough:** None. **E. Freeman:** None. **R. Clements:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.12/FF17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Canadian society of multiple sclerosis

Title: Increased expression of specific NKG2D ligand in a mouse model of multiple sclerosis

Authors: *L. LEGROUX, S. VERSTRAETEN, G. DEBLOIS, A.-N. MOHEBIANY, D. BEAUSEIGLE, N. ARBOUR;
CRCHUM, Univ. De Montréal, Montreal, QC, Canada

Abstract: **Introduction**: Multiple sclerosis (MS) is considered the prototypic inflammatory disease of the central nervous system (CNS). Lesions in the CNS of MS patients are characterized by myelin sheath destruction, oligodendrocyte death, axonal and neuronal damage, and activation of glial cells. It is well established that the immune system participates in the pathogenesis of MS. Nevertheless, the contribution of specific immune mediators to injury

remains to be defined. NKG2D is an activating receptor expressed by numerous immune effector cells including subsets of CD8 and CD4 T cells. NKG2D binds to various ligands (NKG2DL), which include RAE-1(alpha to epsilon), MULT1, and H60 (a-c) in mice. NKG2DL are induced by environmental triggers (e.g. inflammation) suggesting that these proteins alert the immune system to abnormal cells. Our laboratory has previously showed that oligodendrocytes express at least one NKG2DL in MS lesions but not in control brains. Moreover, CD8 T cells in MS lesions are detected in close proximity to NKG2DL expressing cells. We have also established that disruption of the NKG2D-NKG2DL interaction inhibits killing of human oligodendrocytes by activated immune effector cells in vitro. One group showed that blockade of NKG2D diminished disease severity in the MS mouse model: experimental autoimmune encephalomyelitis (EAE). Overall, these results imply that NKG2D-NKG2DL interaction can contribute to the pathogenesis of MS and its animal model EAE. However, whether specific NKG2DL are upregulated by CNS cell subsets in vivo during the development of EAE and could potentially be targeted is still unresolved.
Methodology: Using well established EAE mouse models, we assessed qualitatively and quantitatively the expression of NKG2D and NKG2DL during different stages of the development of EAE. We used flow cytometry, qRT-PCR, western blot and immunohistochemistry approaches.
Results: We observed that MULT-1, one specific ligand of NKG2D, is upregulated in the CNS of EAE mice and such elevated expression correlates with disease severity. Moreover, MULT-1 is detected on neurons, astrocytes and endothelial cells and is released in the extracellular matrix and cerebrospinal fluid during EAE. In contrast, expression of other ligands does not vary throughout disease. Finally, we also observed a greater proportion of CNS infiltrating CD4 and CD8 T cells expressing NKG2D compared with cells from other organs. Finally, the elevated NKG2D expression on CD8 T cells correlates with augmented effector functions (IFNγ, GM-CSF and granzyme B production). Our results suggest that NKG2D and specific ligands could play a role in the pathogenesis of MS.

Disclosures: L. Legroux: None. **S. Verstraeten:** None. **G. Deblois:** None. **A. Mohebiany:** None. **D. Beauseigle:** None. **N. Arbour:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.13/FF18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: UCLA Laboratories of Neuroendocrinology Training Grant (5T32HD007228)

The Conrad N. Hilton Foundation (#20150232)

The California Community Foundation (#BAPP-15-118094)

The Tom Sherak MS Hope Foundation

Title: Increased gabaergic inhibition through α5-subunit containing $gaba_a$rs contributes to impaired hippocampal synaptic plasticity in eae

Authors: *L. G. KAMMEL[1], W. WEI[2], R. VOSKUHL[3], T. O'DELL[2];
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Abstract: Cognitive impairment affects 40-65% of multiple sclerosis (MS) patients. Using the mouse model of MS (experimental autoimmune encephalomyelitis, EAE), we investigated if disease-induced functional deficits in the hippocampus underlie this impairment. We found that the induction of LTP was significantly reduced in the hippocampal CA1 from EAE mice (fEPSPs were potentiated to 135.9 ± 4.3% of baseline in controls, n = 5, compared to 119.8 ± 2.6% in EAE, n = 6, $p < 0.05$) using 150 pulses of 5 Hz presynaptic fiber stimulation. Also, tonic inhibition mediated by extrasynaptic GABA receptors was strongly enhanced in CA1 pyramidal cells from EAE mice (currents were 0.43 ± 0.07 pA/pF in controls, n = 13 and 0.76 ± 0.06 pA/pF in cells from EAE mice n = 28, $p < 0.01$). Since enhanced tonic inhibition could oppose the induction of LTP, and tonic inhibition in the hippocampal CA1 is mediated by α5-subunit (GABRA5) containing $GABA_A$Rs, we next investigated whether blocking the GABRA5-sensitive tonic current could ameliorate the deficit in LTP during EAE. Consistent with this hypothesis, 150 pulses of 5 Hz stimulation in the presence of the GABRA5 inverse-agonist L-655,705 abolished differences in levels of LTP in slices from control versus EAE mice (fEPSPs were potentiated to 138.4 ± 5.2% of baseline in controls, n = 5, and 136.6 ± 5.5% in slices from EAE mice, n = 5). It had been reported that GABRA5 expression is upregulated in post-mortem hippocampal tissue from MS patients, therefore we next investigated if altered expression of GABRA5 could underlie the enhanced tonic current in EAE. Indeed, GABRA5 plasma membrane (PM) expression was significantly upregulated in the EAE hippocampus (GABRA5/FLOT-1: EAE, n=6, showed 157.9 ± 12.0% expression of controls, 100 ± 8.8%, n=5, $p< 0.01$). Finally, because reduced GABA uptake could also contribute to the enhanced tonic current, we additionally investigated if the PM expression of neuronal (GAT-1) or astrocyte (GAT-3) GABA transporters was altered in EAE. While GAT-1 showed no differential PM expression, GAT3 PM expression was significantly decreased in the EAE hippocampus (GAT3/FLOT-1: EAE, n=6, showed 69.4 ± 6.2% expression of controls, 100 ± 2.8% n=5, $p< 0.01$). Together these data suggest that targeting altered GABAergic neurotransmission, mediated by the dysregulation of α5-subunit containing $GABA_A$Rs and of astrocyte GABA transporters, warrants investigation as a strategy for treating hippocampal-dependent cognitive impairments in MS.

Disclosures: **L.G. Kammel:** None. **W. Wei:** None. **R. Voskuhl:** None. **T. O'Dell:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.14/GG1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: INSERM

ARSEP

Title: molecular MRI reveals pselectin protein as a predictive marker in experimental autoimmune encephalomyelitis

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Abstract: Developing new strategies for detection of disease activity in multiple sclerosis is important to ameliorate the diagnosis and follow-up of this pathology. For that, we used microparticles of iron oxide (MPIO) coupled to an antibody specific to the p-selectin protein and a MRI sequence triggered with the animal breathing. In this study, we aimed to demonstrate that molecular magnetic resonance imaging (MRI) specific to p-selectin protein is able to detect the pathological events that take place in the spinal cord of **chronic and relapsing experimental autoimmune encephalopathy (EAE)** in mice. More interestingly, we show here that this MRI technique can **predict the apparition of EAE**. Further, we used Evans blue ex-vivo optical imaging combined with immunostaining to visualise blood spinal cord barrier (BSCB) opening and immune cell infiltration. We show that with a single injection of microparticles, this innovative MRI technique can reveal the BSCB opening together with the immune cell infiltration. Our data suggest that molecular MRI targeting-p-selectin allows non-invasive measurement of vascular inflammation and thus, could be used as additional information inaccessible to conventionally used MRI techniques.

Disclosures: R.M. Macrez: None. **A. Quesnault:** None. **A. Quesnault:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Support: Conrad N. Hilton Foundation 20150232

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Tom Sherak MS Hope Foundation

NIH Training Grant 5T32HD007228

NIH NRSA predoctoral fellowship 1F31NS096906

Title: Estrogen receptor beta (ERβ) on $CD11c^+$ cells is required for ERβ-ligand mediated neuroprotection during experimental autoimmune encephalomyelitis

Authors: *R. KIM[1,2,3], N. ITOH[3], A. HOFFMAN[3], R. KOVASH[3], R. VOSKUHL[3];
[2]Molecular, Cell. and Integrative Physiol. Grad. Program, [3]Multiple Sclerosis Program and Dept. of Neurol., [1]UCLA, Los Angeles, CA

Abstract: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). EAE is the most widely used animal model of MS and we have previously shown that ERβ-ligand treatment is neuroprotective during disease. Also, ERβ-ligand treatment can exert anti-inflammatory effects on CNS resident microglia and dendritic cells (DC). However, whether these cell types are directly targeted by ERβ-ligand treatment during EAE remains unknown. Here, we used CD11c promoter to generate conditional knockout (CKO) mice of ERβ (CD11c-CKO-ERβ) using the *Cre-loxP* system. CD11c is known to be a marker for DCs, however, activated myeloid derived cells including microglia and macrophages may also express CD11c during EAE. We induced EAE in CD11c-CKO-ERβ mice and wildtype littermate control (CD11c-WT-ERβ) mice, with or without ERβ ligand treatment to determine if ERβ expression on these cells was critical for treatment-induced neuroprotection. Our results demonstrated that CD11c-WT-ERβ mice treated with ERβ-ligand had significant improvement in clinical disease, as well as protection from axonal and myelin loss in spinal cord, with no significant effect on levels of immune cell infiltration, and that this protection from disease was lost in CD11c-CKO-ERβ mice. Next we investigated whether ERβ-ligand treatment may have induced phenotypic changes (activation; MHCII, polarization; M1 – iNOS, M2 – Arg1) on CNS resident and infiltrated myeloid derived $Iba1^+$ cells during EAE. We observed that ERβ-ligand treated CD11c-WT-ERβ mice had reduced MHCII expression and M1 polarization, with no effect on M2 polarization, and that this ERβ ligand treatment effect on phenotype was lost in CD11c-

CKO-ERβ mice. Together these data indicate that protective effects of ERβ-ligand treatment during EAE are mediated through ERβ on $CD11c^+$ cells, and this entails reducing MHCII activation and iNOS expression on CNS resident and infiltrated myeloid derived $Iba1^+$ cells.

Disclosures: R. Kim: None. **N. Itoh:** None. **A. Hoffman:** None. **R. Kovash:** None. **R. Voskuhl:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.16/GG3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R37 NS041435

NMSS Collaborative Center Award

Title: Oligodendrocyte lineage tracing in a multiple sclerosis mouse model—a cuprizone-fed mouse transferred with myelin-reactive th17 cells.

Authors: *J. JIN, M. SMITH, D. HEO, M. POUDEL, D. TOSI, E. BAXI, D. BERGLES, P. A. CALABRESI;
Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Multiple sclerosis (MS) is an acquired inflammatory demyelinating disease of the central nervous system. It is known that failure of remyelination can lead to irreversible axonal damage. However, the mechanisms underlying remyelination failure are still unclear. Inflammation is a key factor in the initiation and persistence of MS. Understanding the response of oligodendrocyte precursor cells (OPC) during demyelination/remyelination in an inflammatory context may help identify strategies to facilitate remyelination and protect axons from damage.
In this study we used a PDGFaR-Cre:ROSA26-YFP double-transgenic mouse, which express inducible YFP in PDGFaR+ cells, to examine OPC proliferation, viability, and differentiation during demyelination and remyelination in an inflammatory environment. Mice were fed cuprizone (or normal chow) for four weeks followed by a transfer of myelin specific Th17 T-cells (or vehicle) (Baxi, et al.). Mice were recombined after three weeks of cuprizone treatment and were sacrificed 1 or 2 weeks after cuprizone cessation and cell transfer.
As previously shown, Black-Gold II staining showed more severe demyelination in the corpus callosum at 1 week post-transfer Th17 cells and impaired remyelination at 2 weeks in Cup+Th17

mice vs Cup-only. A week post-transfer, there were abundant CD3+ T-cells infiltrating the brain which were still proliferating at 2 weeks post-transfer in Cup+Th17 mice. These infiltrated T cells were more clustered at areas of impaired remyelination. The numbers of different oligodendrocyte lineage traced YFP+ populations were compared in the corpus callosum of Cup+Th17 mice with those of Cup-only mice. At 1 week, there were no CC1+ cells in the corpora callosa of both Cup+Th17 and Cup-only groups. YFP+ cells and PDGFaR+ OPC cells were significantly decreased in Cup+Th17 mice vs Cup-only mice. At 2 weeks, total YFP+ cells and mature oligodendrocytes (CC1+ and CC1+/YFP+cells) were decreased in Cup-Th17 mice. The percentage of pre-myelinating oligodendrocytes was higher in Cup+Th17 mice, indicating these cells did not develop into mature oligodendrocytes. These pre-myelinating oligodendrocytes might have their differentiation arrested and/or be targeted for apoptosis upon initiating differentiation by the infiltrating T-cells and their accompanying inflammation. The investigation into how these inflammatory cues and immune cells are involved in the remyelination failure of Cup+Th17 mice is ongoing. Our ultimate goal is to find targetable pathways to reduce the local CNS inflammation mediated inhibiton of OPCs in order to facilitate remyelination and thus protect axons from degeneration.

Disclosures: **J. Jin:** None. **M. Smith:** None. **D. Heo:** None. **M. Poudel:** None. **D. Tosi:** None. **E. Baxi:** None. **D. Bergles:** None. **P.A. Calabresi:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.17/GG4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Lundbeck grant, R151-2013-14896

Title: Role of RhoA in T cell adhesion and migration in an experimental model of multiple sclerosis

Authors: *A. MANRESA ARRAUT, H. HASSELDAM, F. F. JOHANSEN;
Biotech Res. & Innovation Ctr., Kobenhavn N, Denmark

Abstract: Experimental autoimmune encephalomyelitis (EAE), an animal model of brain inflammation, shares essential clinical and pathological features with Multiple Sclerosis (MS). The main trigger of central nervous system (CNS) inflammation and demyelination in EAE is an activation of CD4+ T-cells in the periphery followed by infiltration into the CNS. The small GTPase RhoA regulates processes which are crucial for immune cell function such as actin

assembly, cytoskeleton plasticity and stress fiber formation, all involved in cell adhesion and migration.
The primary aim of this project is to investigate whether specifically knocking out the small GTPase RhoA in T-cells will impact their ability to induce disease. For this purpose we induced EAE in C57BL/6 mice lacking RhoA expression in T-cells. Our results show that $RhoA^{-/-}$ mice experience decreased disease incidence and severity, compared to their wild-type littermates. Mice heterozygous for the deletion of RhoA in T-cells ($RhoA^{+/-}$) present a similar disease pattern as their wild-type littermates. However, a milder form of the disease is observed, indicating that the presence of only one allele does not provide T-cells with a fully functional RhoA activity. Secondarily we plan to further investigate the role of RhoA in T-cells. We have set up an *in vitro* blood brain barrier model that will allow us to study the migratory capacity of the $RhoA^{-/-}$ T-cells across the brain endothelium. In parallel we have set up a proliferation assay to study if the lack of RhoA impacts T-cell proliferation after TCR stimulation, using both unspecific CD3-stimulation and MOG-specific stimulation. A thorough analysis of the integrin expression and cytokine production will be conducted in $RhoA^{-/-}$ T-cells using flow cytometry, multiplex immunoassays and cell binding assays.
Taken together, our data suggest that RhoA activity in T-cells plays an important role in onset and progression of EAE, which might be due to its role in leukocyte adhesion and migration.

Disclosures: **A. Manresa Arraut:** None. **H. Hasseldam:** None. **F.F. Johansen:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2PO1HD023315

RO1EY019012

P30ES005022

NMSS RG4257B4/1

RO1NS036647

PHS grant MH104800

Title: Strain differences in sensitivity to cuprizone induced demyelination

Authors: *Q. YU[1], R. HUI[1], Y. HUANG[2], A. KUSNECOV[3], C. F. DREYFUS[2], R. ZHOU[1]; [1]Chem. Biol., [2]Neurosci. and Cell Biol., [3]Psychology, Rutgers, Piscataway, NJ

Abstract: Multiple Sclerosis (MS) is a severe neurological disorder caused by demyelination of the central nervous system (CNS) and affects about 2.5 million people worldwide. However the molecular mechanisms underlying the pathogenesis of the disease remain unclear. We investigated the role of genetic differences in contributing to demyelination by using the cuprizone toxicity model with mice of different genetic background (CD1 and C57BL/6). We demonstrate using luxol fast blue (LFB) staining that exposure to diet containing 0.2% cuprizone treatment resulted in less severe demyelination in CD1 mice than C57BL/6 mice. With continuous cuprizone administration, demyelination in CD1 mice was not prominent until after 7 weeks of treatment, in contrast to C57BL/6 mice, in which demyelination was already prominent at week 4 of exposure. Concomitantly, immunohistochemical analysis of the cuprizone treated brain sections of the corpus callosum overlying the fimbria fornix demonstrated significantly more GST-pi+ oligodendrocytes in the CD1 mice relative to C57BL/6 mice. Moreover, CD1 mice exhibit fewer GFAP+ astrocytes and Iba1+ microglia. In order to rule out the issue of potential difference in diet consumption between strains, we measured food intake per body weight between CD1 mice and C57BL/6 mice and report no significant differences. Thus, genetic background factors appear to influence the susceptibility to cuprizone induced demyelination, and our findings might provide new insight into the detailed mechanism of the demyelination process.

Disclosures: Q. Yu: None. **R. Hui:** None. **Y. Huang:** None. **A. Kusnecov:** None. **C.F. Dreyfus:** None. **R. Zhou:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS-019108

4UH-2 TR000918-03

3UH3TR00918-03S1

The University of Chicago Innovation Fund

Title: Interferon gamma-stimulated human dendritic cells produce promyelinating exosomes and replicate rodent studies

Authors: *K. M. PUSIC[1], L. WON[2], A. D. PUSIC[2], R. P. KRAIG[2];
[1]Dept. of Neurol., [2]Univ. of Chicago, Chicago, IL

Abstract: Multiple sclerosis (MS) and migraine are interrelated healthcare burdens which involve considerable negative impact. Spreading depression (SD), the underlying cause of migraine with aura and a well-accepted animal model for migraine, occurs with transient demyelination (1) and increased oxidative stress (2). This is similar to MS where damage to myelin and oxidative stress prevent remyelination.
We have developed a novel cell-based therapy - microRNA-containing (i.e., especially miR-219) exosomes from IFNγ-stimulated dendritic cells (SDC-Exos) - that for the first time remyelinates damaged rat brain (3) and prevents SD (4), perhaps by reducing oxidative stress. Given the uncertainty in translating rodent studies to human therapeutics, we are testing the possibility of producing human SDC-Exos to recapitulate the effects seen from rodent SDC-Exos .
Exosomes were produced from human dendritic cells (DCs) cultured from three sources: peripheral blood, cord blood and bone marrow. Our methods involve selective derivation of adherent immature DCs, which are then stimulated by IFNγ for collection of conditioned media three days later. Identity of DCs was confirmed by morphology and immunostaining (i.e., $CD11b^-$ for macrophages and $CD11c^+$ for DCs). Exosome isolation was confirmed via electron microscopy (i.e., ~100 nm vesicles) and western blot for surface marker CD63. All three sources were successfully differentiated into DCs and produced exosomes. We found that human bone marrow-derived SD-Exos triggered a significant increase (~170%) in myelin basic protein, a marker for myelin, three days after application to rat hippocampal brain slice cultures. This is consistent with the ~122% increase seen in these cultures after application of rat SD-Exos (3) that reached a peak of 150% at five days before returning to normal by seven days (Pusic AD, unpublished observations). Human SD-Exos also contained a significant (> 1000-fold increase versus exosomes derived from unstimulated human DCs) level of miR-219, a microRNA that is necessary and sufficient for promoting oligodendrocyte precursor cell differentiation (5). Also, SDC-Exos showed no evidence of microgliosis.
These results support the feasibility and utility of producing SDC-Exos from autologous and heterologous **human** DC sources as a novel therapeutic to mitigate the impact of neurodegenerative disorders involving myelin dysfunction and oxidative stress, without producing negative inflammatory sequelae.
1. Pusic AD et al, Exptl Neurol, 2015; 2. Grinberg YY et al, J Neurochem, 2012; 3. Pusic SD et al, J Neuroimmunol, 2014; 4) Schumer J et al, Soc Neurosci Abst , 2015; 5) Dugas JC et al, Neuron, 2010.

Disclosures: **K.M. Pusic:** None. **L. Won:** None. **A.D. Pusic:** None. **R.P. Kraig:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.20/GG7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Anti-neuroinflammatory effect of VB037 in experimental autoimmune encephalomyelitis model

Authors: *H. LI, Y.-S. LO, K.-H. CHANG;
Neurol. Dept., Chang-Gung Mem. Hosp., Guishan Dist., Taoyuan, Taiwan

Abstract: Multiple sclerosis is an immune-mediated demyelinating disease of human central nervous system. The experimental autoimmune encephalomyelitis (EAE) model is the most commonly used experimental model for resembling multiple sclerosis. In the in-vitro study we prove the anti-neuroinflammatory effects of one testing drug, VB037, which is extracted from Chinese medicine. In the current study, we induct a monophasic form of EAE model in C57BL/6 mouse via immunization with myelin oligodendrocyte glycoprotein (MOG_{35-55}). We then initiate drug testing via intraperitoneal injection for 14 days. Drug responses are monitored by weight changes and EAE score (0-5) for clinical severity. Our preliminary data shows that VB037 has significant effects in prevention of weight loss and in EAE score severity deterioration. Western blot analysis and immunohistochemistry stain with anti-Iba1 antibody, a macrophage/microglia-specific calcium-binding protein, also confirm the same results. In conclusion, we demonstrate that using the EAE mouse model, VB037 may have anti-neuroinflammatory effects in postponing the disease onset and progression.

Disclosures: H. Li: None. **Y. Lo:** None. **K. Chang:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Guangdong Natural Science Foundation #2016A030313105

National Natural Science Foundation of China #81501171

Title: AdipoR agonist, AdipoRon, decreases lipid accumulation and ameliorates the functions of myelin-laden macrophages

Authors: *X. SUN[1], Q. ZHOU[1], H. XIANG[1], A. LI[1], C. QIN[1], X. CHEN[2], Y. REN[3]; [1]Guangdong-Hong Kong-Macau Inst. of CNS Regeneration, Jinan Univ., Guangzhou City, China; [2]Inst. of Inflammation and Diseases, the First Affiliated Hosp. of Wenzhou Med. Univ., Wenzhou, China; [3]Dept. of Biomed. Sciences, Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Myelin-laden macrophages, resulting from phagocytosis of myelin debris by infiltration of bone marrow-derived macrophages, are abundantly present in demyelinating CNS injuries and diseases, such as spinal cord injury and multiple sclerosis. The accumulation of myelin debris in these macrophages induces neuroinflammation by stimulating the expression of pro-inflammatory factors, and impairing their phagocytic capacity. Lipids are major components of myelin debris; therefore, efficient efflux of myelin lipid may help ameliorate macrophage functions. Adiponectin is a protein hormone involved in metabolic processes that can increase cholesterol efflux from oxLDL-treated macrophages. In this study, we found that AdipoRon, a novel small molecule agonist of adiponectin receptors, could effectively decrease myelin lipid accumulation and suppress foam cell formation in myelin-laden macrophages in both time- and dose-dependent manners. Such effects were partially mediated by increased levels of ABCA1, but not ABCG1. AdipoRon also promoted M2 polarization of myelin-laden macrophages. Furthermore, AdipoRon treatment *in vitro* could ameliorate the functions of myelin-laden macrophages by inhibiting the production of pro-inflammatory factors as well as enhancing their phagocytosis of apoptotic cells. These data suggest that AdipoRon may be a promising therapeutic approach for the treatment of macrophage-mediated neuroinflammation in demyelinating CNS disorders.

Disclosures: **X. Sun:** None. **Q. Zhou:** None. **H. Xiang:** None. **A. Li:** None. **C. Qin:** None. **X. Chen:** None. **Y. Ren:** None.

Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Normandy Graduate School

Title: Interactions between stress-induced modifications of intestinal inflammation and the occurrence of EAE relapses.

Authors: *A. FOURNIER[1], M. NEUNLIST[2], D. VIVIEN[1], R. MACREZ[1], F. DOCAGNE[1]; [1]INSERM U919, Caen, France; [2]INSERM U913, Nantes, France

Abstract: Clinical observations have shown a link between acute stress and the occurrence of relapses in MS patients. Here, we show a repeated acute stress model (water avoidance stress) induces a twofold increase in the incidence of relapses in relapsing-remitting EAE in mice, together with an increase in intestinal permeability. We hypothesize that physiological stress induces an increase in intestinal permeability which would provide an immunological context favouring the occurrence of relapses. To test this, we developed an innovative molecular MRI imaging of gut inflammation. This technique allowed us to show that an intestinal inflammation occurs during EAE relapses. Further, we used a model of gut inflammation induced by dextran sodium salt (DSS) in EAE to check if intestinal inflammation can induce an increase in relapse incidence. Our data suggest that treating gut inflammation (pre/probiotics, micronutrients…) could prevent relapse occurrence and limit the effect of stress. This study should also help taking into account stress management in patient care. In addition, the innovative MRI techniques developed in this study could bring new advances in the diagnosis and prognosis of MS relapses by targeting gut inflammation.

Disclosures: A. Fournier: None. **M. Neunlist:** None. **D. Vivien:** None. **R. Macrez:** None. **F. Docagne:** None.

Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH 1R01NS094522-01

NIH R21 NS084303-01A1

FISM 2012/R/02

Title: Opposite functions of microglial and monocyte/macrophagial TNFR2 in EAE pathogenesis: the good versus the bad

Authors: *H. GAO[1], M. DANZI[1], C. S. CHOI[2], M. TAHERIAN[1], C. DALBY-HANSEN[3], D. G. ELLMAN[3], P. M. MADSEN[3], J. L. BIXBY[1], V. P. LEMMON[1], K. L. LAMBERTSEN[3], R. BRAMBILLA[1];
[1]Univ. of Miami, Miami, FL; [2]Columbia Univ., New York, NY; [3]Univ. of Southern Denmark, Odense, Denmark

Abstract: In multiple sclerosis (MS), soluble tumor necrosis factor (TNF) has been attributed detrimental functions, whereas transmembrane TNF promotes neurorepair primarily by activating TNF receptor 2 (TNFR2). Here we investigate the role of TNFR2 in microglia and peripheral monocytes/macrophages using novel cell-specific conditional knockouts in experimental autoimmune encephalomyelitis (EAE), a model of MS. We show that microglial TNFR2 ablation leads to early onset of EAE, with increased leukocyte infiltration in the central nervous system (CNS), T cell activation, and demyelination. TNFR2-ablated microglia shows a more pro-inflammatory phenotype, with dysregulation of genes controlling innate immunity and host defense. Conversely, monocyte/macrophagic TNFR2 ablation results in EAE suppression, with impaired peripheral T cell activation, and reduced CNS T cell infiltration and demyelination. Our work uncovers a dichotomy of function for TNFR2 in myeloid cells, with microglial TNFR2 providing protective signals to contain disease, and monocyte/macrophagic TNFR2 driving immune activation and EAE initiation. The complexity of TNFR2 function must be taken into account when targeting TNFR2 for therapeutic purposes in neuroinflammatory diseases.

Disclosures: H. Gao: None. **M. Danzi:** None. **C.S. Choi:** None. **M. Taherian:** None. **C. Dalby-Hansen:** None. **D.G. Ellman:** None. **P.M. Madsen:** None. **J.L. Bixby:** None. **V.P. Lemmon:** None. **K.L. Lambertsen:** None. **R. Brambilla:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Mitoxantrone prevents disease relapse in a rat model of multiple sclerosis

Authors: *E. ANDRIAMBELOSON, J. BINDLER, C. NEVEU, L. BOURGOIN, L. GORJ, B. HUYARD, N. KADOUCI, F. LAUGA, E. POIRAUD, S. WAGNER;
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Abstract: Mitoxantrone (Novantrone) is an immunosuppressive drug that impacts both T and B cell proliferation. In 2000, mitoxantrone was approved by the FDA for the treatment of multiple sclerosis (MS). Indeed, clinical data suggests that the drug markedly reduces relapse rate in patients with very active relapsing remitting MS (relapses reduced by up to 70-80%) and significantly reduces the accrual of disability over 2 years in patients with relapsing remitting MS. However, there is no animal data showing the beneficial effect on the disease relapse when mitoxantrone treatment is initiated only after the occurrence of the first attack. The present study investigates the effect of mitoxantrone in a rat model of relapsing remitting experimental allergic encephalomyelitis. In this model, the disease relapse is observed after a short (4-5 days) and partial recovery period following the first attack. The severity of clinical disability during the relapse is comparable to that observed during the first attack. Continuous daily treatment with 0.5 mg/kg mitoxantrone initiated after the remittance from the first attack fully suppressed the course of disease relapse. The accrued disability score over the disease relapse period was significantly reduced. For comparison, preventive daily treatment with 0.5 mg/kg, initiated before the appearance of any clinical signs of the first attack, gave a 90% of the accrued disability score during the relapse period. Furthermore, the authors wanted to determine whether mitoxantrone might have a direct effect on CNS inflammation. The results showed that mitoxantrone does not prevent LPS-induced neuronal inflammation and death in a mixed glia-neuron culture which suggests the beneficial effect of the drug is not likely to be driven by a direct attenuation of inflammation on CNS-resident cells. A more plausible target of mitoxantrone for the herein observed in-vivo effect thus might be an immunomodulation of peripheral immune response.

Disclosures: E. Andriambeloson: None. **J. Bindler:** None. **C. Neveu:** None. **L. Bourgoin:** None. **L. Gorj:** None. **B. huyard:** None. **N. Kadouci:** None. **F. Lauga:** None. **E. Poiraud:** None. **S. Wagner:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.25/GG12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant

NMSS Grant

Title: Therapeutic estrogen receptor beta (ERβ) ligands modulate peripheral cytokines and may be responsible for remyelination in a mouse model of multiple sclerosis.

Authors: *H. KARIM[1], J. HASSELMANN[1], N. YASUI[2], J. KATZENELLENBOGEN[2], S. TIWARI-WOODRUFF[1];
[1]Biomed. Sci., Univ. of California, Riverside, Riverside, CA; [2]Univ. of Illinois, Urbana, IL

Abstract: Estrogen receptor β (ERβ) ligands have been shown to induce remyelination in different mouse models of multiple sclerosis (MS). Recent work using the chronic myelin oligodendrocyte glycoprotein experimental autoimmune encephalomyelitis (MOG-EAE), has shown that different ERβ ligands (diarylpropionitrile (DPN), Indazole chloride (IndCl), WAY 202041 (WAY)) all demonstrate remyelination, but show differential immune effects in the periphery. Previous studies have shown that treatment with DPN confers remyelination without significant effects on inflammation, whereas IndCl confers neuroprotection and inhibits inflammation. Recent results from our group have shown similar neuroprotection with WAY without obvious effects on inflammation. Here, we wanted to compare the effect of these different ligands on peripheral cytokines and CNS immune cells. MOG-EAE was induced by standard protocols from the Tiwari-Woodruff lab. Flow cytometry and peripheral splenocyte cytokine analysis were performed on vehicle, prophylactic 17β-estradiol (E2) and therapeutic ERβ ligand-treated age and sex matched C57Bl/6 female mice. Vehicle treated EAE mice showed disease onset between 7-10 days. Positive control pre-E2 treated animals did not show any clinical disease. Unlike DPN-treated animals (similar onset of disease as vehicle-treated group), IndCl and WAY treatment delayed onset of EAE clinical symptoms. Day 21-post EAE flow analysis revealed an increasing trend in resting microglia and a significant decrease in macrophages in pre-E2 but not IndCl, WAY and DPN treated groups. A significant decrease in Th1 and Th17 CNS cells was observed only in pre-E2 treated groups. None of the treatments modified either the Th2 or the Treg CNS population. A decrease in splenic macrophages was observed with pre-E2 treatment. Interestingly, an increase in macrophages was observed in all ERβ ligand treated groups. Peripheral cytokine analysis revealed a reduction of IFNγ, interleukin (IL)-17, GM-CSF and IL-2 with an increase of IL-10 expression in IndCl and WAY treated EAE groups, similar to pre-E2 group. An interesting result was a significant increase of the chemokine CXCL1 (involved in oligodendrocyte proliferation and recruitment) in all ERβ ligand treated groups, with no change in E2 and vehicle groups. The present study indicates an intricate interplay between the peripheral and central immune systems that seems to be responsible for the remyelinating effects of ERβ ligand treatment during EAE. More studies including conditional gene knockouts are needed to address the relationship between immune modulation and neuroprotection by ERβ ligands.

Disclosures: H. Karim: None. **J. Hasselmann:** None. **N. Yasui:** None. **J. Katzenellenbogen:** None. **S. Tiwari-Woodruff:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Program#/Poster#: 424.26/GG13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit Review

Title: The contribution of dysfunctional hnRNP A1 and anti-hnRNP A1 antibodies to MS pathogenesis

Authors: *H. SALAPA, S. LEE, Y. SHIN, M. C. LEVIN;
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: It is becoming evident that inflammation and neurodegeneration occur simultaneously but independently during multiple sclerosis (MS). Heterogeneous nuclear ribonuclear protein A1 (hnRNP A1) is an RNA binding protein (RBP) that shuttles between the nucleus and cytoplasm to modulate RNA metabolism. Nuclear import of hnRNP A1 is mediated by its nuclear localization sequence, M9. RBPs, including hnRNP A1, have been implicated in the pathogenesis of other neurological disorders. Common features of these disorders include altered mRNA transport functions, redistribution of RBPs, and the formation of pathogenic stress granules. Our lab has found that MS patients, in contrast to controls, make autoantibodies to hnRNP A1-M9. We hypothesized that hnRNP A1 might exhibit pathogenic features similar to other neurological disorders using *in vitro* and *in vivo* models of MS. Furthermore, we sought to determine whether anti-hnRNP A1-M9 antibodies might contribute to disease pathogenesis. For *in vitro* experiments, SKNSH cells were treated with T_h17 cytokines (IL-17A, IL-23) and examined for hnRNP A1 mislocalization. For *in vivo* experiments, female C57BL/6J mice were induced with MOG_{33-55} experimental autoimmune encephalomyelitis (EAE). At the first sign of disease, mice were injected with either PBS, IgG2b isotype control or anti-hnRNP A1-M9 antibodies (100 mcg/mL X 3), which recognize the immunodominant epitope of hnRNP A1 in MS patients. Animals were scored based on disease severity. In contrast to untreated SKNSH cells, cells exposed to T_h17 cytokines showed mislocalization of endogenous hnRNP A1 from the nucleus to cytoplasm, a sign of cellular stress. *In vivo*, animals exposed to anti-hnRNP A1-M9 antibodies exhibited worse disease ($p<0.05$) and more frequent spasticity, a common symptom seen in MS patients. Interestingly, spastin, a known RNA binding partner of hnRNP A1 under normal conditions and causative factor in hereditary spastic paraplegia, was altered in EAE. Specifically, spastin protein levels in the brain were significantly decreased in anti-hnRNP A1-M9 as compared to control, PBS, and IgG animals ($p=0.034$). These data suggest that dysfunctional hnRNP A1 may contribute to MS pathogenesis in a manner similar to other RBPs in neurological diseases. Pro-inflammatory cytokines induced hnRNP A1 mislocalization.

Additionally, anti-hnRNP A1-M9 antibodies worsened disease, led to the development of spasticity in the hind limbs of animals, and altered protein levels of spastin in the brain. This suggests that anti-hnRNP A1-M9 antibodies could play a causative role in the development of spasticity in autoimmune diseases of the CNS, including MS.

Disclosures: **H. Salapa:** None. **S. Lee:** None. **Y. Shin:** None. **M.C. Levin:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.27/GG14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Temporal changes in the glial response mechanism in the Cuprizone/Rapamycin model of Multiple Sclerosis

Authors: ***M. MADDIE**[1], D. CHMURA[1], S. LUNN[1], H. BATTAPADY[1], S. MEDICETTY[1], B. TRAPP[2];
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Abstract: Multiple sclerosis (MS) is a chronic immune mediated disease with primary demyelination, formation of glial scars, and progressive neurodegeneration in the central nervous system. The cuprizone-rapamycin (C/R) mouse model is a non-immune mediated, toxic model of MS demonstrating extensive demyelination in both white and grey matter with axonal injury. It has been previously demonstrated that C/R model has robust microglial and astrocytic activation within the corpus callosum by 4 weeks of injury. In this study, we quantified the glial response to injury in the corpus callosum, hippocampus, and cortex to elucidate the region specific temporal response to demyelination. In the corpus callosum, we show that there is approximately 50% loss of oligodendrocytes following 2 weeks of C/R treatment, and almost total loss of oligodendrocytes by 6 weeks. Concurrent with early oligodendrocyte loss we observe a significant increase in microglial activation (Iba1 staining) in the corpus callosum by 2 weeks of C/R (2.7 fold increase in Iba1 when compared to WT control). Peak Iba1 response was seen at 4 weeks of C/R prior to subsequent decrease in staining at 6 and 12 weeks of demyelination (5.7, 5, and 4.5 fold increase in staining compared to WT control respectively). There was no increase in astrocyte marker GFAP at 2 weeks of C/R; however, GFAP activation steadily increases over 4, 6 and 12 weeks of demyelination (5.07, 5.5, 7.6 fold increase in staining compared to WT control respectively). Furthermore, examination of phagocytic macrophages (Mac2) demonstrates a gradual increase in staining over the 12-week paradigm (0.5, 0.8. 1.5 and 2.4. fold increase in staining compared to WT control respectively). In contrast to corpus callosum,

hippocampus and cortex, both of which are demyelinated in this model showed little activation of astrocytes or microglia. These observations demonstrate unique temporal signatures of glial activation in response to C/R treatment. Furthermore, this study highlights a differential glial response to demyelination in white and grey matter. This model can be extremely valuable to study therapeutic interventions that target glial responses in MS.

Disclosures: **M. Maddie:** A. Employment/Salary (full or part-time): Renovo Neural. **D. Chmura:** A. Employment/Salary (full or part-time): Renovo Neural. **S. Lunn:** A. Employment/Salary (full or part-time): Renovo Neural. **H. Battapady:** A. Employment/Salary (full or part-time): Renovo Neural. **S. Medicetty:** A. Employment/Salary (full or part-time): Renovo Neural. **B. Trapp:** A. Employment/Salary (full or part-time): Cleveland Clinic Foundation. F. Consulting Fees (e.g., advisory boards); Renovo Neural.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

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Program#/Poster#: 424.28/HH1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS084206

Title: Ketone body esters as a therapeutic strategy for canavan disease

Authors: *A. P. APPU, P. ARUN, J. R. MOFFETT, J. K. KRISHNAN, A. M. NAMBOODIRI; C-2069, Anatomy, Physiol. and Genet., Uniformed Services Univ. of Hlth. Sci., Bethesda, MD

Abstract: Canavan disease (CD) is a rare neurological disease resulting from genetic defects that manifest as a progressive neurodegenerative disease leading to paralysis and death, usually between 3 and 10 years of age. There is no effective treatment at the present time. CD is caused by mutations in the gene for the enzyme aspartoacylase (ASPA), which produces free acetate from the concentrated brain metabolite *N*-acetylaspartate (NAA). NAA is synthesized in neurons, but ASPA is expressed primarily in oligodendrocytes, and evidence indicates that neurons transfer NAA to oligodendrocytes for acetyl CoA synthesis. Since acetyl CoA is a key building block for myelin lipid synthesis and other critical developmental functions such as gene regulation through histone acetylation, we postulated that the inability to enzymatically catabolize NAA leads to an acetate deficiency in oligodendrocytes during postnatal CNS myelination, resulting in oligodendrocyte death and defective myelin lipid synthesis. Previously we tested the hypothesis that dietary acetate supplementation during postnatal myelination would reduce the severe phenotype associated with ASPA deficiency using the tremor rat model of CD.

Glyceryl-triacetate (GTA), a hydrophobic acetate source, was administered to tremor rats starting 7 days after birth, and administration was continued in food and water after weaning. Significant improvements were observed in motor performance and brain galactocerebroside content in tremor rats treated with GTA. Further, the characteristic brain vacuolation associated with CD was modestly reduced by the treatment. In our efforts to test additional acetate/acetyl CoA sources alone and in combination to improve outcomes further, we have tested a ketone body ester since ketone bodies form a major source of acetyl CoA in the brain during development. The treatment was done essentially as described for GTA, but in the Nur 7 mouse model of CD. Significant improvement in motor functions as measured by rotarod balance was observed in the treated mice. Pathological analysis is in progress to determine if the characteristic vacuolation of ASPA deficiency was reduced. Importance of the ongoing studies lies in the fact that CD has no current treatment and remains a fatal disease which is devastating to the affected families. In contrast to gene therapy, acetate (acetyl CoA) replacement therapy is a simple biochemical approach, which is safe, inexpensive and convenient for use in CD infants.

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Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.29/HH2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIGMS grant T32 GM007507

NIH grant RO1-NS37570

AHA 152500022

Title: Neuronal antigen specific T cells modulate CNS inflammation during autoimmunity

Authors: *A. RAYASAM[1,2,3], M. HSU[1,2,3], M. DALLMANN[1], J. KIJAK[1], N. ZINDL[1], M. SANDOR[1,2], Z. FABRY[1,2,3];
[1]Univ. of Wisconsin - Madison, Madison, WI; [2]Pathology, [3]Neurosci. Training Program, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), which results in axonal demyelination and oligodendrocyte dysfunction. Most of the mouse models of MS such as experimental autoimmune encephalomyelitis (EAE) involve an

immunization to initiate CD4+ T cells to become autoreactive towards myelin. These models mainly evaluate the physical motor dysfunctions observed in MS patients, however, there is strong evidence that higher order neurological and cognitive deficits also occur as well. In order to address the role of anti-neuronal immune responses during MS, we generated transgenic mice using the Nestin specific promoter to localize an EGFP-tagged fusion protein containing ovalbumin (OVA) antigenic peptides in Nestin+ cells (Nestin-OP mice). Using this model, we tested how anti-OVA peptide-specific OT-I (CD8+) and OT-II (CD4+) T cells contribute to disease progression during EAE. Interestingly, adoptive transfer of OT-I and OT-II T cells into Nestin-OP mice significantly reduced EAE clinical scores compared to littermate controls. This suggests that in Nestin-OP mice, the Nestin recognizing T cells become anti-inflammatory or the T cell response steers toward non-motor Nestin+ brain areas. In order to understand the mechanism, we analyzed the cytokine producing potential of the OT-I and OT-II T cells and evaluated the inflammatory and synaptic environment in Nestin+ brain areas in Nestin-OP and littermate mice immunized with EAE. Our results suggest that autoreactive T cells targeted towards non-myelin antigens can influence CNS function independent of EAE score suggesting that therapies targeted towards brain antigen specific T cells deserve more attention for treating MS.

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Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.30/HH3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Kent State University

Title: Erythropoietin upregulates brain hemoglobin expression and levels of H3K4me3

Authors: ***N. K. SINGHAL**, K. ALKHAYER, J. SHELESTEK, R. CLEMENTS, E. FREEMAN, J. MCDONOUGH;
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Abstract: Multiple sclerosis (MS) is a neuro-inflammatory and demyelinating disease. Downregulation of neuronal mitochondrial gene expression and activity have been reported in several studies of MS. We have previously shown that hemoglobin-β can signal to the nucleus of neurons and upregulates H3K4me3, a histone mark involved in regulating cellular metabolism

and differentiation. The present study was undertaken to evaluate the effect of erythropoietin (EPO) and upregulation of hemoglobin-β on mitochondrial associated neuroprotection. We found that administering EPO (5000IU/kg of mice intraperitoneally) to mice upregulated brain hemoglobin expression, levels of H3K4me3, expression of complex III, complex V, mitochondrial respiration, and NAA levels. To extrapolate these results to MS, we treated mice with cuprizone (3% in food diet) for six weeks to induce demyelination with or without EPO. Demyelination was measured by magnetic resonance imaging (MRI) and NAA concentration was measured by HPLC as a marker of neuronal mitochondrial activity. We found upregulation of NAA in cuprizone-EPO treated mice as compared to cuprizone only. These data suggests that EPO regulates neuronal mitochondrial activity by modulating H3K4me3 levels.

Disclosures: N.K. Singhal: None. **K. Alkhayer:** None. **J. Shelestek:** None. **R. Clements:** None. **E. Freeman:** None. **J. McDonough:** None.

Poster

424. Neuroprotection in Models of Immune Mediated Demyelination

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 424.31/HH4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intrathecal delivery of primary progressive MS cerebrospinal fluid induces behavioral deficits and spinal cord pathology in mice

Authors: *J. K. WONG, M. ALAHIRI, S. S. SADIQ;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Multiple sclerosis (MS) is characterized by inflammatory demyelination, astrogliosis and axonal loss in the CNS, which leads to progressive clinical disability. The majority of patients initially present with relapsing-remitting MS (RRMS), where periods of neurological decline are interspersed with periods of clinical stability. Approximately 10-15% of MS patients have primary progressive MS (PPMS), which manifests as continual progression of clinical disability from disease onset. Although disease-modifying therapies that target the immune system have been effective for RRMS patients, they do not halt disease progression in PPMS patients, suggesting that inflammation may have a more minor contribution to PPMS pathophysiology. Indeed, PPMS lesions tend to contain fewer inflammatory cells and atrophy is predominantly observed in the cervical spinal cord. Currently, there is no animal model that encompasses these characteristics of PPMS.
Here, we sought to develop an animal model of PPMS that exhibits characteristic pathology in the upper regions of the spinal cord. Intrathecal injections of cerebrospinal fluid (CSF) obtained

from either PPMS or RRMS patients were administered to mice. Control mice were injected with saline or CSF obtained from healthy individuals. Mice underwent laminectomies at cervical levels 4 and 5 to expose the underlying spinal cord, and the fluids were injected under the dura mater into the subarachnoid space. Behavioral deficits were assessed by evaluating forelimb reaching and gripping, as well as tail rigidity at multiple time points post-injection. Mice injected with PPMS CSF exhibited significantly impaired forelimb function and increased tail flaccidity as compared to controls, as well as mice injected with RRMS CSF. Mice were perfused at 1 day post-injection (DPI), 3 DPI, and 7 DPI. Spinal cords were post-fixed in 4% paraformaldehyde overnight, cryoprotected in 30% sucrose, then cryosectioned for histological analyses. Spinal cords from mice injected with PPMS CSF exhibited evidence of astrogliosis at all time points examined, as revealed by increased GFAP immunostaining in the dorsal spinal cord. Luxol fast blue staining showed areas of demyelination only in mice injected with PPMS CSF. In contrast, there were no significant differences in Iba-1 immunostaining between treatment groups, suggesting a minimal contribution of microglial activation towards the development of behavioral deficits and spinal cord pathology following intrathecal PPMS CSF injections.

Disclosures: J.K. Wong: None. **M. Alahiri:** None. **S.S. Sadiq:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.01/HH5

Topic: C.08.Stroke

Support: Leverage Research and Development Award, Research and Development Corporation

Heart and Stroke Foundation Canadian Partnership for Stroke Recovery

Canada Research Chairs Program

Title: Differences in audio and visual working memory during computerized cognitive rehabilitation in chronic stroke survivors

Authors: *E. M. WALLACK[1], L. P. KELLY[1], A. J. DEVASAHAYAM[1], T. CHATTERJEE[1], M. B. DOWNER[1], J. MCCARTHY[1], G. A. ESKES[2], B. ABRAHA[1], S. M. M. HASAN[1], A. R. CHAVES[1], H. D. WISEMAN[1], J. DAWE[1], M. PLOUGHMAN[1];
[1]Med., Mem. Univ., St. John's, NL, Canada; [2]Dalhousie Univ., Halifax, NS, Canada

Abstract: Background: Persistent deficits in executive function, attention and learning can have detrimental effects on stroke recovery. Working memory involves a number of prefrontal-

parietal networks, which can be sensory modality specific. There is evidence of age related differential decline in audio and visual working memory and Jaeggi and group found some advantage for processing visual material compared to auditory in an n-back working memory task in a group of young and older healthy adults. These differences raise the question of whether visual and auditory working memory would respond the same way to training in a chronic stroke population and differential response to training may impact the outcomes and generalizability to other tasks and functions. Thus, we compared training performance in visual and auditory domains on a similar working memory task in stroke.
Methods: Participants (n=14) were more than 6 months post-stroke from enrolment. Each participant underwent a 10 week (30 minutes, 3 times a week) computerized adaptive dual n-back task training protocol that required them to respond to sequentially presented paired audio and visual stimuli. Measures of stroke severity (National Institutes of Health Stroke Scale; NIH) and cognitive function (Montreal Cognitive Assessment. MoCA) were also collected at baseline. Wilcoxon Signed-ranks tests were used to compare groups and correlation using Pearson's.
Results: Twelve males and 2 females participated (age 65.1 ± 9.7yrs). They had moderately severe stroke (NIH 7.9, SD=4.8) involving the right (n=7), left (n=6) or both (n=1) hemispheres. At week 1 and week 10 participants performed significantly better on audio tasks than visual tasks ($p= 0.001$, $p=0.03$ respectively). Participants showed significant improvement in accuracy over the 10 weeks of training in both audio ($p=0.001$) and visual ($p=0.001$) modalities. Rate of change in auditory and visual performance did not differ during the 10 weeks of training ($p=0.9$). Stroke severity was not correlated with rate of change or with week 1 and week 10 auditory and visual scores. Performance on audio tasks at week 1 was significantly positively correlated with baseline MoCA scores $r(12) = 0.767$, $p=0.01$.
Conclusion: Participants showed improvements over the 10 weeks of training in auditory and visual modalities suggesting learning is feasible after stroke. While participants advanced at a similar pace for both modalities, the auditory advantage persisted at week 10 of training. This auditory advantage may have implications for training generalization and outcome in stroke.

Disclosures: E.M. Wallack: None. **L.P. Kelly:** None. **A.J. Devasahayam:** None. **T. Chatterjee:** None. **M.B. Downer:** None. **J. McCarthy:** None. **G.A. Eskes:** None. **B. Abraha:** None. **S.M.M. Hasan:** None. **A.R. Chaves:** None. **H.D. Wiseman:** None. **J. Dawe:** None. **M. Ploughman:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.02/HH6

Topic: C.08.Stroke

Support: Leverage Research and Development Award, Research and Development Corporation,

Heart and Stroke Foundation, Canadian Partnership for Stroke Recovery

Canada Research Chairs Program

Title: Maximal exercise does not increase brain derived neurotropic factor (BDNF) in chronic stroke survivors: association with resting energy metabolism and peak oxidative capacity

Authors: *L. P. KELLY, A. J. DEVASAHAYAM, E. M. WALLACK, B. ABRAHA, J. MCCARTHY, M. B. DOWNER, S. M. M. HASAN, F. A. BASSET, M. PLOUGHMAN;
Fac. of Med., Mem. Univ., St. John's, NL, Canada

Abstract: Background: Brain derived neurotrophic factor (BDNF) plays a crucial role in the recovery of the injured brain after stroke. Serum BDNF is considered a surrogate biomarker for brain plasticity. Furthermore, the beneficial effects of exercise on peripheral energy metabolism may be mediated through BDNF signaling. The purpose of this study was to determine the effect of maximal exercise on serum BDNF and its association with resting and maximal oxidative capacity.
Methods: Eighteen non-diabetic chronic stroke survivors (>6months) with no significant contraindications to maximal graded exercise testing (GXT) were recruited. Participants were first subjected to a resting metabolic rate measurement using a metabolic cart and flow-through canopy to determine whole-body resting energy production (EP) and the relative contributions from lipid and glucose oxidation (Lox and Gox, respectively). On a separate day, participants had resting blood samples drawn just prior to and immediately after a GXT for later analysis of serum BDNF. The GXT was performed on a total body recumbent stepper to exhaustion while heart rate (HR), ventilation (VE) and expired air were sampled for rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using a facemask and metabolic cart. Serum BDNF was compared pre and post-exercise using t-test; and correlation of variables using Pearson's.
Results: Participants (11F, 7M) were 68±9 years of age, and 34±25 months post-stroke. Although BDNF levels were relatively high at rest prior to exercise (30.7±19.2 ng/ml), post-exercise values were not significantly different from resting (33.1±16.1; p=0.56). A very low oxidative capacity was observed during the GXT (16.1±5.5 ml min^{-1} kg^{-1}), however, resting energy metabolism was within ranges expected based on age and gender (EP=1.0±0.2 kcal min^{-1}; Lox=68.7±22.6 %; Gox=14.3±21.6%). There were no significant correlations between resting or post-exercise BDNF and resting or maximal oxidative capacity.
Conclusion: The current analysis suggests that exercise does not increase serum BDNF concentrations beyond that observed at rest in chronic non-diabetic stroke survivors. People with stroke who have very low oxidative capacity may have blunted BDNF response to maximal exercise. Our findings further elucidate the usefulness of serum BDNF as a marker of plasticity.

Disclosures: L.P. Kelly: None. **A.J. Devasahayam:** None. **E.M. Wallack:** None. **B. Abraha:** None. **J. McCarthy:** None. **M.B. Downer:** None. **S.M.M. Hasan:** None. **F.A. Basset:** None. **M. Ploughman:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.03/HH7

Topic: C.08.Stroke

Support: Leverage Research & Development Award , Research & Development Corporation

Heart and Stroke Foundation , Canadian Partnership for Stroke Recovery

Canada Research Chairs Program

Title: Can circulating bdnf levels discriminate high from low impairment in chronic stroke survivors?

Authors: *B. ABRAHA, E. M. WALLACK, L. P. KELLY, A. J. DEVASAHAYAM, T. CHATTERJEE, S. GRANTER-BUTTON, J. MCCARTHY, M. PLOUGHMAN;
Med., Mem. Univ., St. John's, NL, Canada

Abstract: Background: Stroke rehabilitation aims to restore lost cognitive and motor skills which requires neuroplasticity. Recent research has implicated Brain-Derived Neurotrophic Factor (BDNF) as a precursor of neuroplasticity. There is debate whether or not peripheral (serum) BDNF could be a useful biomarker of recovery and plasticity in stroke. We investigated whether serum levels of BDNF were related to measures of physical and cognitive neurological impairment in stroke survivors.
Methods: All participants (n= 35) suffered a haemorrhagic or ischemic stroke greater than 6 months. They performed a graded maximal exercise test, with blood collection before and after. We used National Institute of Health Stroke Scale (NIH) to measure the global level of neurological impairment; Chedoke-McMaster stage of combined leg and foot recovery to measure degree of motor recovery and cognition using Montreal Cognitive Assessment (MOCA) and Ravens Matrices (test of fluid intelligence). Serum BDNF concentrations expressed in ng/ml, were determined using manufacturer's protocol (R&D Systems ELISA). The sample was split into high (>5 NIH) and low (≤ 5) impairment compared using ANOVA. BNDF levels pre and post exercise were compared to outcomes using correlation analysis.
Results: There were 23 males, 65.1 years of age ($\pm$9.9) and 12 females, 65.5 years of age ($\pm$8.8). Patients with greater stroke severity (>5 NIH) had higher serum BDNF [$F(1, 33) = 10.157$, $p = 0.003$]. In addition, serum BDNF fold-change ($R=0.44$; $p< 0.05$), and BDNF raw change ($R=0.40$; $p<0.05$) were significantly correlated with fluid intelligence (Raven's Matrices). Global cognition (MOCA) was also significantly correlated with baseline BDNF ($R=-0.41$, $p <0.05$). While physical impairment (Chedoke-McMaster) was not significantly related to baseline serum BDNF or the responsiveness of BDNF.

Conclusion: Paradoxically, patients with greater stroke severity had higher resting serum BDNF whereas the change in BDNF (as a result of exercise) was related to fluid intelligence. Also individuals with greater baseline levels of BDNF had lower global cognition. These findings suggest that the relationship between serum BDNF and neuroplasticity is complex but in this sample, BDNF was a more robust marker of cognitive rather than physical impairment.

Disclosures: B. Abraha: None. **E.M. Wallack:** None. **L.P. Kelly:** None. **A.J. Devasahayam:** None. **T. Chatterjee:** None. **S. Granter-Button:** None. **J. McCarthy:** None. **M. Ploughman:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.04/HH8

Topic: C.08.Stroke

Support: Leverage Research & Development Award , Research & Development Corporation

Heart and Stroke Foundation , Canadian Partnership for Stroke Recovery

Canada Research Chairs Program

Title: Is BDNF response to maximal exercise associated with performance in cognitive rehabilitation training in chronic stroke survivors?

Authors: *H. D. WISEMAN, M. B. DOWNER, B. ABRAHA, E. M. WALLACK, T. CHATTERJEE, L. P. KELLY, A. J. DEVASAHAYAM, A. R. CHAVES, J. MCCARTHY, M. PLOUGHMAN;
Mem. Univ., St. John's, NL, Canada

Abstract: Background: Cognitive recovery post stroke is highly variable, and currently there are few ways to predict response to cognitive training in chronic stroke survivors. Increased circulation of key neurotrophic factors such as brain derived neurotropic factor (BDNF) may be a key link to cognitive recovery post stroke. Several exercise paradigms have been shown to up-regulate circulating levels of BDNF. It is currently unclear whether differences in BDNF levels from exercise are associated with differences in response to cognitive training in chronic stroke survivors. **Methods:** All participants experienced a haemorrhagic or ischemic stroke more than 6 months pre-enrollment. Participants underwent a 10 week intervention consisting of 30 minutes of exercise followed by 30 minutes of cognitive training, 3 times a week. A graded maximal fitness test was performed before the intervention, with blood collection before and after

exercise. The blood was left to coagulate for 30 minutes, centrifuged for 10 minutes at 1500rpm and plasma was extracted for later analysis using a BDNF ELISA kit. Cognitive training consisted of a computerized sequential adaptive dual n-back task (N-IGMA). The cognitive score was calculated by combining both level and accuracy of the task. Classification of responders and non-responders was determined by averaging peak working memory capacity scores. Participants who improved 35% or greater from their week 1 score were classified as "responders" and those who did not were coded as "non-responders". **Results:** Responders (n=6) had a mean age of 64.2 (SD±10.72) and non-responders (n=5) had a mean age of 64.6 (SD±9.017). Pre-exercise BDNF levels between responders (M=51.86, SD±19.37) and non-responders (M=72.68, SD±28.07) were not significantly different (F=2.116, p=0.180). Responders mean change in BDNF (20.51 ng/mL (SD±6.54)) as a result of exercise is significantly higher than BDNF than in non-responders (-11.05 ng/mL (SD±12.90)) (F=5.305, $p<0.05$). **Conclusions:** Our findings suggest that acute change in BDNF levels due to exercise may indicate ability to respond to cognitive training in those living with chronic stroke. Individuals who demonstrate a greater up-regulation of BDNF through exercise may be able to use the higher concentration of factors to help improve in cognitive performance soon thereafter; whereas individuals with less up-regulation may have less ability to respond to cognitive training. Further rehabilitation programs could use serum BDNF levels as a way to determine those who are more likely to respond to paired exercise-cognition training post-stroke.

Disclosures: H.D. Wiseman: None. **M.B. Downer:** None. **B. Abraha:** None. **E.M. Wallack:** None. **T. Chatterjee:** None. **L.P. Kelly:** None. **A.J. Devasahayam:** None. **A.R. Chaves:** None. **J. McCarthy:** None. **M. Ploughman:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.05/HH9

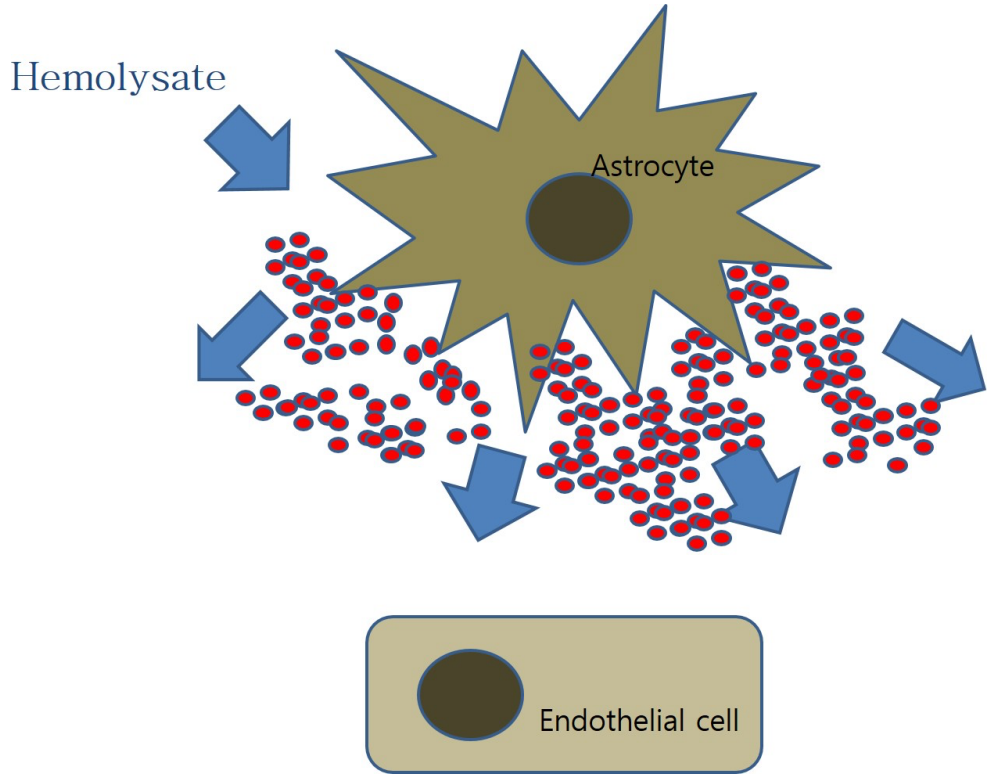
Topic: C.08.Stroke

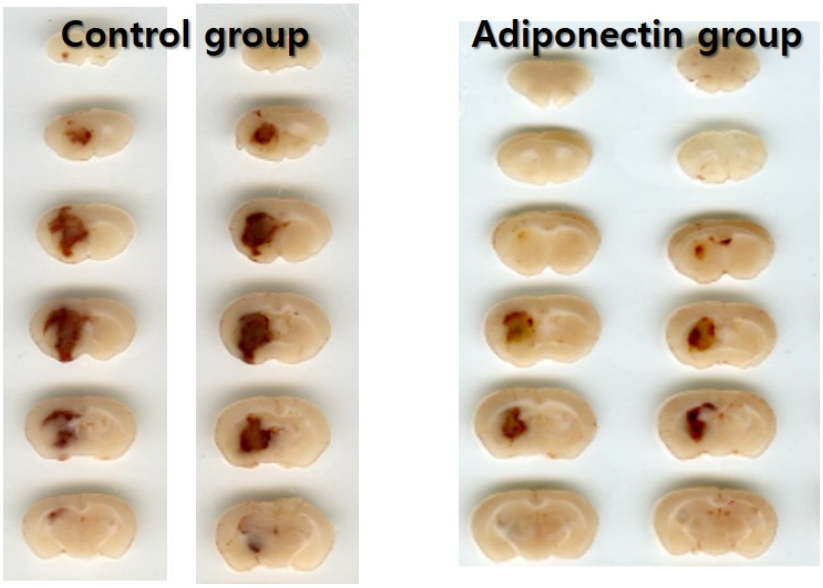
Title: Anti-inflammatory activity of adiponectin function on BBB after intracerebral hemorrhage

Authors: *X. YANG[1], H. JANG[2], Y.-J. KIM[3], I.-Y. CHOI[3], S.-H. LEE[4], B.-W. YOON[4]; [1]The neurology depertment, The Seoul Natl. Univ. Hosp., seoul city, Korea, Republic of; [2]Dept. of cardiology, [3]Col. of Med. and Neurosci. Res. Inst., [4]Dept. of Neurol., Seoul Natl. Univ., seoul, Korea, Republic of

Abstract: *Background* Obesity is the increased prevalence of diseases. Adipose tissue associated with obesity secreted many cytokines, such as adiponectin, one of the most abundant adipokine

with anti-inflammatory effects. The clinical significance of adiponectin in cardiology has been extensively identified, but in stroke remains controversial. *Method and results* To investigate the role of adiponectin in the intracerebral hemorrhage (ICH), we subjected collagenase-induced male imprinting control regions (ICR) mice of ICH before adiponectin treatment. ICR mice exhibited large hematoma and edema after ICH, however, adiponectin preconditioned group (APN-ICR) significantly attenuated these damages. Furthermore, APN-ICR mice showed decreased the inflammation and MMP activation. In culture of astrocytes stimulated by hemolysate to mimic the hemorrhage situation, inflammatory activations were significantly reversed by adiponectin. Endothelia cells, which were exposed to conditioned media from astrocytes stimulated by hemolyste, significantly increased expression tight junction proteins. However, adiponectin abolished disorganization of them. *Conclusions* Taken together, these results suggest that adiponectin exerts an important neurovascular protective effects on mice of ICH and support the mechanism of crosstalk between reactive astrocytes and endothelia wherein adiponectin inhibiting the inflammatory activities and enhance the blood-brain barrier.





Disclosures: **X. Yang:** None. **H. Jang:** None. **Y. Kim:** None. **I. Choi:** None. **S. Lee:** None. **B. Yoon:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

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Program#/Poster#: 425.06/HH10

Topic: C.08.Stroke

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Activity-dependent regulation of neurogenesis after stroke

Authors: ***H. LIANG**[1], S. T. CARMICHAEL, 90066[2];
[1]Neurol., Univ. of California Los Angeles Dept. of Neurol., Los Angeles, CA; [2]UCLA, Los Angeles, CA

Abstract: Stroke is the leading cause of adult disability. Post-stroke neurogenesis has been implicated in repair and functional recovery in stroke; however the cellular and molecular mechanism that regulates neurogenesis after stroke remains unclear. Although activity has been shown to regulate neurogenesis during development in normal brain, how modulation of activity affects neurogenesis after stroke is not fully elucidated. In this study, we employed forced use of the paretic forelimb that mimics constraint-induced movement therapy (CIMT) as a physical

modulation of activity after stroke. We found that forced use of paretic forelimb contralateral to the stroke site enhanced neuroblast migration and neural progenitor proliferation in the peri-infarct cortex at post-stroke day 14; neuronal differentiation was increased on post-stroke day 60.Interestingly,when forced use was performed in the hindlimb contralateral to the stroke site, proliferation and neuroblast migration to the peri-infarct cortex was largely abolished and long term neuronal differentiation significantly decreased with a surprising substantial increase in glial differentiation. These data suggest that activity-induced neurogenesis after stroke is circuit specific and is dependent on regions of modulation. To further demonstrate the role of activity in a cell type specific manner, we utilized the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) as a pharmacological approach to modulate both neuronal and glial activity in the peri-infarct region. We found that post-stroke neuroblast migration significantly increased when cortical CaMKII-expressing neurons were activated with DREADD stimulation. Interestingly, selective use of the inhibitory DREADD in astrocytes decreased post-stroke neuroblast migration. These data suggest that post-stroke neurogenesis is modulated through both neuronal and glial activity in the peri-infarct regions. Finally, neuronal tracing techniques including BDA labeling and rabies-virus based monosynaptic tracing are ongoing to address whether activity regulates integration of adult-born neurons into the injured cortex circuitry.

Disclosures: H. Liang: None. **S.T. Carmichael:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.07/HH11

Topic: C.08.Stroke

Support: Japan Society for the Promotion of Science, KAKENHI, 26350599

Title: Customary exercise prevents the poststroke memory dysfunction by constitutive elevation of hippocampal BDNF

Authors: *N. HIMI[1], N. OKABE[1], E. NAKAMURA-MARUYAMA[1], H. TAKAHASHI[2], T. KOGA[2], K. NARITA[1], O. MIYAMOTO[1];
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Abstract: Exercise in the poststroke stage is known to facilitate the recovery from memory and motor dysfunction. We have showed that the spatial memory function was recovered by the elevation of hippocampal brain-derived neurotrophic factor (BDNF) only in the early stage after

stroke. In the present study, we further investigated the preventive effects of customary exercise on memory and motor impairment induced by stroke. In this study, two different stroke models were employed to confirm the preventive effect on different symptom and severity. Microsphere (MS) embolism model showed spatial memory dysfunction and internal capsule infarction model showed motor dysfunction. Rats were forced to run on a treadmill with mild strength (15m/min, 30min/day) for 7 days as the exercise (Ex group). At the 8th day after the beginning of exercise, 3,000 particles of MSs (ϕ45μm) were injected via right internal carotid artery of rats (MS group) or photosensitive dye was injected into artery and 488 nm laser was irradiated to internal capsule by optic fiber (IC group). Non-exercise group (NE group) and sham operated groups were also examined as control groups. The Morris water maze test (MS group) and rotarod test (IC group) were performed at 8 days after the onset of stroke. BDNF concentration in transected hippocampus (MS group) and thalamus (IC group) were measured at 0, 4, 7 days before and 4 and 7 days after the onset of stroke by ELISA.
BDNF concentration was elevated by exercise and decreased after the completion of exercise in both MS- and IC- Ex group. The memory functions in the MS-Ex group was significantly improved compared with the MS-NE group. IC-Ex group showed no significant motor function recovery.
These results suggest that the constitutive BDNF elevation in hippocampus by customary exercise might prevent the spatial memory dysfunction after stroke.

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Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.08/HH12

Topic: C.08.Stroke

Support: Maryland Industrial Partnerships Phase 1: Physical Rehabilitation Software System

Title: Kinect-based upper extremity training is effective for individuals with stroke: Outcomes and participants' perspective

Authors: ***W.-W. LIAO**[1], S. MCCOMBE WALLER[1], R. FELDMAN[1], J. WHITALL[1,2]; [1]Physical Therapy Rehabil. Sci., Univ. of Maryland Baltimore, Baltimore, MD; [2]Univ. of Southampton, Southampton, United Kingdom

Abstract: The low-cost virtual reality system such as Microsoft Kinect has become an increasingly popular tool for continued stroke rehabilitation outside of traditional rehabilitation. Despite its popularity, the usability, therapeutic methods and participants' perspective towards use of computer-based training such as the Kinect system remains largely unaddressed. The purpose of this study was to investigate the effectiveness of Kinect-based upper extremity (UE) training on arm impairments and functional performance in individuals with stroke in order to determine its applicability in a clinical/home setting. In addition, we examined stroke survivors' experience regarding Kinect-based training. **Methods:** Ten individuals (mean age 62.5±9.06 years) with chronic hemiparesis (7Lt,3Rt) were recruited from the local community. Subjects participated in Kinect-based UE training three times a week with a target duration of 4-5 weeks. To simulate the home/clinic environment, the therapist guided participants at the initial three sessions, then withdrew input for the remaining of the training including interface with the system to start the exercise program. Outcome measures included Fugl-Meyer UE assessment (FM-UE), Wolf Motor Function Test (WMFT), Active Range of Motion for shoulder and elbow (AROM), the hand function portion of Stroke Impact Scale (SIS) and Confidence in Arm and Hand Function (CAHM). Participants' experience and feedback toward use of Kinect UE training was assessed using a structured questionnaire and a semi-structured interview. **Results:** Significant improvement was found in FM-UE, WMFT, AROM as well as the Hand function of SIS post-training ($p<.05$). Eight out of 10 individuals reported increased use of the paretic arm in daily activities in the interview. Ninety percent of individuals found the training engaging and easy to follow reporting that the visual and auditory feedback were useful in training. All recognized the importance of the individualized modification made by the therapists at the beginning of training. **Conclusion:** We demonstrated that four weeks of Kinect-based UE training was able to provide clinically important and self-perceived improvements in arm function for chronic stroke survivors. Individualization of the Kinect exercises by therapists, at least in the first few sessions, appears important for use in the clinics/home setting.

Disclosures: W. Liao: None. **S. McCombe Waller:** None. **R. Feldman:** None. **J. Whitall:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.09/HH13

Topic: C.08.Stroke

Support: BMBF Grant 031 5581B

BMBF Grant 01GQ0923

DFG Research Unit Grant For 1738 (HHDP)

Title: Stroke induced brain morphological changes and the influence of physical exercise

Authors: S. GULL[1], S. SCHMIDT[1,2], K.-H. HERRMANN[3,2], C. FRAHM[1], J. REICHENBACH[3,2], C. GASER[1,2,4], *O. W. WITTE[1,2,5];
[1]Hans Berger Dept. of Neurol., [2]Brain Imaging Ctr. Jena, [3]Inst. of Diagnos. and Interventional Radiology, [4]Dept. of Psychiatry, [5]Biomagnetic Center, Hans Berger Dept. of Neurol., Friedrich Schiller Univ. Jena, Jena, Germany

Abstract: *Objective:* Stroke represents one of the leading causes of adult disability and recovery is highly challenging for most survivors. Early restorative therapies like appropriate physical exercise are shown to improve the outcome in motor function. It is believed that exercise improves spontaneous intrinsic mechanisms of recovery but how the brain reorganize following stroke and how this reorganization is influenced by therapies is not fully understood. Here we used a longitudinal approach to analyze the influence of acrobatic training on dynamic changes of brain morphology following motor cortex lesions in young adult rats by employing magnetic resonance imaging (MRI) and deformation-based morphometry (DBM). *Method:* Experimental stroke was induced in the right motor cortex of male Wistar rats (3 months old) by Photothrombosis (PT). One group of animals was subjected to daily acrobatic training starting immediately following stroke for 8 weeks with slowly increasing difficulty whereas the remaining animals recovered spontaneously. T2-weighted 3D MR images were acquired longitudinally at baseline and 1, 2, 4, 8 weeks following PT and processed by DBM. The performance of paw placement during locomotion and the outcome of recovery were analyzed by using the ladder rung walking task. *Results:* Stroke impaired the performance of contra-lesional paws. Our acrobatic training regime did not influence the dynamic of lesion volume but it significantly improved the outcome of recovery. DBM analysis revealed a pattern of training independent structural reorganization of the brain. Most of these changes were restricted to the initial recovery phase and the lesioned hemisphere whereas the training enhanced their amplitudes but not overall dynamics. In addition, acrobatic training evoked structural changes in a second cluster of brain areas including the putamen and the lesion surrounding cortex. *Conclusion:* Acrobatic training improves the final outcome of motor function recovery after motor cortex stroke - reflected by whole brain structural reorganization which is most prominent in the early recovery phase. Further analyses are needed to understand the recovery process as a whole and to explore which specific types of plasticity takes place in different remote areas.

Disclosures: S. Gull: None. **S. Schmidt:** None. **K. Herrmann:** None. **C. Frahm:** None. **J. Reichenbach:** None. **C. Gaser:** None. **O.W. Witte:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.10/HH14

Topic: C.08.Stroke

Support: NIH R01

NIH R21

Title: Short bouts of exercise before ischemic stroke ameliorate behavioral and histological outcomes by enhancing angiogenesis

Authors: *S. PIANTA, H. NGUYEN, S. MASHKOURI, D. AUM, X. KAYA, N. TAJIRI, S. ACOSTA, J.-Y. LEE, C. V. BORLONGAN;
Dept. of Neurosurg. and Brain Repair, Univ. of South Florida Col. of Med., Tampa, FL

Abstract: Stroke remains a significant unmet clinical need with limited therapeutic options. Exercise has been the shown to afford therapeutic benefits in stroke patients, but the mechanism of action is still unknown. The peculiar feature of ischemic stroke is the interruption in brain blood circulation, therefore finding strategies to maintain the potency of the cerebrovasculature may provide a therapeutic effect against stroke. The present study assessed the effects of short bouts of exercise prior to experimental stroke induction, and characterized in vivo the cerebral blood flow, concomitant motor functions, and immunohistochemical markers of angiogenesis and vasculogenesis. Initially, adult Sprauge-Dawley rats were exposed once to short bout of exercise (30-minute or 60-minute of forced running wheel), then they were subjected to transient intraluminal occlusion of the middle cerebral artery (MCAo). Separate cohorts of non-exercised stroke rats served and no stroke rats served as controls. Cerebral blood flow (CBF) was evaluated by laser Doppler at baseline (prior to MCAo), during MCAo, and during reperfusion. Behavioral test using the elevated body swing test was conducted at baseline, day 0 (day of stroke), and at days 1 and 3 after stroke. Results revealed significant elevations of CBF during reperfusion in 60-minute exercised rats compared to 30-minute exercised rats and controls. Moreover, the animals that received 60-minute exercise displayed improved motor performance than 30-minute exercise and non-exercised rats. Histological analysis revealed that exercised rats exhibited a reduction of the infarct size area and increased number of the live cells in the peri-infarct area, specifically this trend follows the duration of the exercise. Immunofluorescence staining intensity revealed increased levels of angiogenesis markers Ang-2 and VEGFR-2 and EPC marker CD34+ in exercised groups compared with controls. These results suggest that prophylactic exercise improves cerebrovascular integrity and function, in that exercise-induced facilitation of angiogenesis may attenuate the debilitating stroke outcomes.

Disclosures: **S. Pianta:** None. **H. Nguyen:** None. **S. Mashkouri:** None. **D. Aum:** None. **X. Kaya:** None. **N. Tajiri:** None. **S. Acosta:** None. **J. Lee:** None. **C.V. Borlongan:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.11/HH15

Topic: C.08.Stroke

Support: Vancouver Island Health Authority Collaborative Research Grant

Heart and Stroke Foundation of Canada (BC & Yukon)

Natural Sciences and Engineering Research Council of Canada

Title: Effects of repetitive passive ankle stretch applied with a pneumatic robot on soleus h-reflex excitability

Authors: *S. NOBLE[1,3,4], G. E. P. PEARCEY[1,3,4], C. QUARTLY[5], P. E. ZEHR[1,3,2,4]; [1]Exercise Science, Physical & Hlth. Educ., [2]Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada; [3]Rehabil. Neurosci. Lab., Victoria, BC, Canada; [4]Intl. Collaboration On Repair Discoveries, Vancouver, BC, Canada; [5]Collaborative Spasticity Program, Queen Alexandra Hosp., Vancouver Island Hlth. Authority, Victoria, BC, Canada

Abstract: Neurotrauma can result in spasticity which impairs motor control and walking ability. Spasticity is characterized by hyperactive stretch and electrically evoked Hoffmann (H-) reflexes. Therapeutic stretching is used in spasticity management, but long-term physiotherapy services are expensive and inaccessible to the majority of spastic patients. Commercial devices that provide stretch without a therapist have recently been developed and may be useful, however quantified effects on spinal cord excitability are unknown. H-reflex excitability in 13 neurologically intact participants (9 male and 4 female; age 19-28) was assessed after 30 minutes of gentle passive dorsiflexion stretching induced by a pneumatic robot. H-reflexes evoked in the soleus muscle and ankle range of motion were examined before, during, and after gentle stretch. The pneumatic robot moved the ankle through 91.9 ± 7.3% of maximal active range of motion. The stimuli delivered to evoke H-reflex recordings occurred at ankle joint angle of 88.8 ± 7.5^{o}, corresponding to 77.6 ± 8.2% of active range of motion. H-reflex amplitudes were significantly different (52.4 ± 50% change; $p<0.05$) between pre-stretching and the mid-session sampling in 9 participants and between pre- and post-stretching in 11 of 13 participants (37.1 ± 37.3% change).

These data suggest that 30 min of pneumatic robot-induced gentle passive stretching at the ankle can significantly alter spinal cord reflex excitability.

Disclosures: **S. Noble:** None. **G.E.P. Pearcey:** None. **C. Quartly:** None. **P.E. Zehr:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.12/HH16

Topic: C.08.Stroke

Title: Dysfunctional Notch 3 signaling inhibits the mediation of beneficial effects of physical activity and enriched environment on adult neurogenesis in a transgenic CADASIL mouse model

Authors: *C. KLEIN[1], S. SCHREYER[1], F. E. KOHRS[1], P. EL HAMOURY[1], A. PFEFFER[1], T. MUNDER[1], F. EHRET[2], G. KEMPERMANN[2], B. STEINER[1];
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Abstract: The theory of a "vascular niche" related to adult neurogenesis describes the interaction of neural stem or precursor cells and endothelial cells of cerebral blood vessels. The hippocampus, where new neurons are generated for integrating new spatial memory, is a highly vascularized niche. For CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), the most common hereditary form of stroke and vascular dementia, a dysregulated adult hippocampal neurogenesis has been suggested as a potential mechanism for the manifestation of dementia. Previous work has shown that mice overexpressing Notch 3 with a CADASIL mutation display reduced cell proliferation and survival of newborn neurons at 12 but not 6 months of age. In transgenic controls overexpressing wild type Notch 3, cell proliferation and survival of newborn neurons are already reduced at 6 months. It has been demonstrated that Notch 3 is expressed in hippocampal precursor cells and maturing neurons and that Notch 3 signaling negatively regulates precursor cell proliferation. This suggests a loss-of-function effect in CADASIL. Here, we aimed to further elucidate the role adult hippocampal neurogenesis plays in CADASIL and how neurogenesis is regulated by Notch 3 as part of the vascular niche. Adult neurogenesis can be robustly stimulated by physical exercise and environmental enrichment. To also investigate the influence of such stimuli on a dysregulated hippocampal neurogenesis in the CADASIL mouse model, animals (female, 8-12 weeks old) were housed in standard (STD), environmentally enriched (ENR) or running wheel cages (RUN) for either 28 days or 6 months. Two transgenic mouse lines were used: Tg88+ mice overexpress the mutant form of the gene and develop CADASIL, while the Tg129+ mouse line overexpresses wild type Notch 3 and serves as control for the mutation. Adult neurogenesis is

reduced in older but not yet in younger Tg129+ mice. RUN and ENR failed to stimulate neurogenesis in both transgenic mouse lines. In Tg88+ mice, this is probably due to decreased physical activity, because they ran far less than controls. In contrast, Tg129+ mice were even more active during 28 days of physical exercise than controls but still lack stimulated neurogenesis. Older CADASIL mice displayed enhanced gliogenesis in the hippocampus indicating an increased inflammatory response due to frequent strokes at this stage. This was reduced by six months of RUN but not ENR. The present results suggest that the beneficial effects of physical exercise on cell proliferation and of enriched environment on cell survival in the hippocampus may depend on Notch 3 signaling.

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Poster

425. Stroke Recovery Activity-Dependent Mechanisms

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Program#/Poster#: 425.13/HH17

Topic: C.08.Stroke

Support: Canadian Partnership for Stroke Recovery

Title: Post stroke bimanual performance relates to corpus callosum properties

Authors: *A. M. AURIAT[1], J. LAU[2], J. K. FERRIS[1], J. L. NEVA[1], L. A. BOYD[1];
[1]Physical Therapy, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background: White matter fractional anisotropy (FA) of corpus callosum (CC) tracts relates to post-stroke motor outcome. However, we do not understand how regional differences in CC structure relate to bimanual motor performance after stroke.
Methods: Diffusion weighted resonance imaging was obtained from all participants. Interhemispheric connections from 5 CC subregions (I-anterior, II-anterior midbody, III-posterior midbody, IV- isthmus, V-splenium) were traced by placing a single seed ROI in the medial midsagittal section of the CC. Tracts were generated from the ROI with constrained spherical deconvolution tractography, and FA values for the entire track were obtained. All diffusion processing was completed in ExploreDTI.
A KINARM robot was used for 3 upper-limb assessments: 1) Arm Matching: the robot moved participants' non-dominant/paretic hand to a target positions and the participant had to mirror match the location with the opposite hand. 2) Object-Hit: participants hit virtual balls moving

towards them with virtual paddles attached to each hand. 3) Object-Hit and Avoid: cognitive demand of the Object-Hit task was increased, by requiring participants to hit two target shapes while avoiding all others. Results are displayed as mean ± SD. Correlations were considered significant at a corrected p-level of < 0.01.
Results: We assessed 25 neurologically intact (NI) individuals (age: 61.7 ±9 .6), and 25 individuals (age: 65.6 ± 8.1) at least 6 months (74.6 ± 14.1 months) following a stroke. Arm Matching: Limb matching error was greater following stroke than in the NI group (8.5 ± 4.5 vs 5.6 ± 2.0%, $p = 0.018$). No CC regions related to performance in arm matching for either group ($p \geq 0.075$). Object Hit: Stroke participants hit significantly fewer targets (177.4 ± 51), than the NI group (251.5 ± 35; $p < 0.001$). For the NI group, total hits correlated with FA of CC region II ($r = 0.527$, $p = 0.007$), but this relationship was not significant for the stroke group ($r = 0.422$, $p = 0.035$). Hit and Avoid: Post-stroke participants made fewer target hits (106.2 ± 25) compared to the NI group (149.3 ± 23, $p < 0.001$). Total hits correlated with CC region II ($r = 0.533$, $p = 0.006$) in the NI group but with regions III ($r = 0.505$, $p = 0.009$) and V ($r = 0.511$, $p = 0.008$) of the stroke group.
Conclusion: Post-stroke bimanual motor function is impaired, and does not relate to the FA of the CC region II (motor). However, at least in the more demanding bimanual task, CC regions III (sensory) and V (temporal/occipital) relate to post-stroke motor performance. Suggesting that bihemispheric sensory and visual processing contributes to bimanual post-stroke motor performance.

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Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.14/II1

Topic: C.08.Stroke

Support: NIH NS42617

NIH NS086272

AHA SDG11780023

Title: Robot-assisted mechanical therapy protects against stroke-induced skeletal muscle injury

Authors: *C. L. RINK, M. BALCH, H. HARRIS, S. GNYAWALI, C. K. SEN, S. KHANNA; Surgery, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: The scientific study of post-stroke physical therapy is challenged in part by a lack of objective tools available to researchers for systematic pre-clinical testing. Here we report first outcomes having developed a Robot-Assisted Mechanical Therapy (RAMT) device to objectively address the significance of mechanical physiotherapy on post-stroke outcomes. Male Wistar rats were subjected to the intraluminal thread method of middle cerebral artery occlusion (MCAO) for 90 minutes. Following stroke, rats received up to 14 days of RAMT therapy (0.5N @ 1Hz over 10mm of stroke-affected gastrocnemius for 30min, RAMT+) or matching anesthesia without therapy (RAMT-). The effects of RAMT on post-stroke sensorimotor function were quantified, and stroke-affected gastrocnemius muscle was evaluated. Compared to controls, RAMT+ significantly improved post-stroke sensorimotor function as measured by AnyMaze^{TM} and TreadScan^{TM} software scoring systems. Examination of mechanotransduction-sensitive pathways revealed RAMT increased brain-derived neurotrophic factor (BDNF) and decreased myostatin expression in stroke-affected gastrocnemius muscle. Outcomes establish a reproducible model to quantitatively assess mechanical therapy for sensorimotor recovery following neurological injury and provide first evidence to support RAMT improvement in post-stroke sensorimotor function.

Disclosures: C.L. Rink: None. **M. Balch:** None. **H. Harris:** None. **S. Gnyawali:** None. **C.K. Sen:** None. **S. Khanna:** None.

Poster

425. Stroke Recovery Activity-Dependent Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 425.15/II2

Topic: C.08.Stroke

Support: University of Auckland FRDF Grant 3709325

Title: Delayed administration of citalopram is associated with long-lasting motor improvements and promotes brain remodelling in an experimental model of stroke

Authors: S. CHEN[1], L. BENNET[2], *A. L. MCGREGOR[3];
[1]Sch. of Pharm., [2]Dept. of Physiol., Univ. of Auckland, Auckland, New Zealand; [3]Sch. of Pharm., Univ. of Otago, Dunedin, New Zealand

Abstract: There is growing clinical and experimental evidence that functional recovery after stroke may be modulated by concomitant CNS medication. Recent evidence suggests treatment with antidepressants may promote, while anti-psychotics may suppress functional recovery. However, the timeframe in which these pharmacological agents can influence stroke recovery is not well understood. This research investigated whether delayed administration of citalopram, used clinically in the management of post-stroke depression, could improve functional recovery in an experimental mouse model of stroke.
CSF1-EGFP mice were subjected to 45 min. occlusion of the middle cerebral artery under isoflurane anaesthesia. Animals were administered citalopram (1mg/kg/day, n=13) or saline (n=12) 3 days after stroke for 4 weeks. Neurological deficits and functional performance in the sticky label removal, staircase, and corridor tests were assessed at 1, 2, 4, 6, and 8 weeks post-stroke. 30μm brain cross-sections were processed for thionin and luxol fast blue histology, and immunofluorescence analyses with GAP43, MAP2, and Cyclin D1.
Ischaemic stroke produced a unilateral impairment in food retrieval in the staircase test. Citalopram-treated animals showed significantly improved impaired forepaw use 1, 2, and 6 weeks post stroke compared to controls in staircase test ($p>0.05$). Improved skilled motor function in citalopram-treated animals was associated with reduced EGFP expression ($p<0.05$) and preservation of white matter preservation in the ipsilateral cortex and corpus callosum respectively. Triple-label immunocytochemistry showed an inverse relationship between the level of EGFP expression relative to MAP2, Cyclin D1, and GAP43 expression in the peri-infarct striatum and cortex.
Administration of citalopram 3 days after stroke reduced brain inflammation and produced long lasting improvements in functional performance. Reduced brain inflammation correlated with increased white matter integrity and upregulation of markers of brain remodelling, suggesting that tissue reorganisation contributes to improved functional recovery in these animals. The extended therapeutic window observed in this study indicates that administration of citalopram in the sub-acute phase may be a useful treatment strategy for the large number of stroke patients who experience delayed hospital admission.

Disclosures: **S. Chen:** None. **L. Bennet:** None. **A.L. McGregor:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.01/II3

Topic: D.02. Somatosensation: Pain

Support: Eisai Inc

Title: Long-term recovery from chemotherapy-induced neuropathy following paclitaxel, ixabepilone, eribulin and vinorelbine in mice.

Authors: *K. M. WOZNIAK[1], Y. WU[2], Y. LIU[3], R. WEINBERG[4], V. CAROZZI[6], G. FUMAGALLI[6], P. ALBERTI[6], B. COOK[7], S. BENBOW[8], B. A. LITTLEFIELD[10], K. NOMOTO[12], L. WILSON[8], M. A. JORDAN[9], S. FEINSTEIN[8], S. ECKLEY[11], C. DEJARDINS[11], G. CAVALETTI[6], J. MANKOWSKI[4], M. POLYDEFKIS[3], B. S. SLUSHER[5]; [1]Johns Hopkins Drug Discovery Core, Johns Hopkins Sch. of Med., Baltimore, MD; [2]Johns Hopkins Drug Discovery Core, [3]Neurology, Neuromuscular Med. and Pathology, [4]Comparative Med. Retrovirus Bio Lab., [5]Johns Hopkins Drug Discovery Core and Dept Neurology, Medicine, Psychiatry and Neurosci., Johns Hopkins Med. Sch. of Med., Baltimore, MD; [6]Exptl. Neurology, Sch. of Med. and Surgery, Univ. of Milano-Bicoca, Monza, Italy; [7]Mol Cell Dev Biol. and Neurosci Res. Inst., [8]Biomolecular Sci. and Engin. and Neurosci Res. Inst. and, [9]Neurosci Res. inst and Dept Mol Cell Dev Biol., Univ. of California, Santa Barbara, Santa Barbara, CA; [10]Global Med. Affairs, Oncology Business Unit, [11]Eisai Res. Insitute, Andover, MA; [12]Oncology Business Group, Eisai Inc, Woodcliff Lake, NJ

Abstract: Chemotherapy-induced peripheral neurotoxicity is often a dose-limiting side effect which can persist following cessation of treatment. We are in the process of systematically evaluating the time course and extent of neuropathy recovery in mice up to six months post dosing for four chemotherapies: eribulin (ERIB), paclitaxel (PAC), ixabepilone (IXA) and vinorelbine (VINO). We are evaluating six independent end-points including nerve conduction/amplitude, sciatic nerve /dorsal root ganglia (DRG) morphology, foot pad intra-epidermal fiber density (IEFD), corneal fiber density, microtubule biochemistry and drug pharmacokinetics. The in-life portion of the study is complete and analyses are underway. Mice received MTD doses of ERIB (1.2 mg/kg), PAC (30 mg/kg), IXA (2 mg/kg), or VINO (11 mg/kg) IV 3 times a week for 2 wks. Nerve conduction deficits for all four chemotherapies were maximal between 1-2 wks after cessation of dosing. After 3-6 mo, deficits largely recovered for ERIB and IXA, but remained significant in PAC mice at 6 mos; 3-6 mo VINO studies are underway. Axonal levels of tubulin and acetylated tubulin in sciatic nerve increased at 24h but returned to normal at two wks after PAC or ERIB. Occasional myelin abnormalities persisted, though less than at 24h and axonal density deficits recovered in ERIB, but not in PAC treated mice. Similar studies with VINO and IXA are ongoing. Preliminary DRG morphological analysis indicated PAC induced neuronal damage out to 3 mo post dose; morphometric analysis confirmed reduction in sensory neuron size. Peripheral nerve fiber degeneration, supported by a decrease in g-ratio, was present in sciatic nerves of PAC and IXA-treated mice at 2 wks, but partially resolved at 3 mo. Despite no severe morphological changes in nerves of ERIB and VINO mice, g-ratios were slightly decreased at 2 wks. Corneal immunostaining with confocal microscopy found acute nerve fiber loss between 1 and 2 wks, with varying degrees of regeneration at 6 mo post dose. IENFD analysis of vehicle mice found a stable mean value of 81.4 fibers/mm. In contrast, PAC treatment caused a monotonic mean decrease to a mean nadir of 12.9 fibers/mm 2 wks post and recovered fully by 6 mo. IXA -treated mice also had a decrement to 28.4 fibers/mm by 2 wks and full recovery by 4 wks. These data indicate that unmyelinated sensory fibers are prominently affected and that PAC-induced axon loss is more

severe than IXA. Analyses of ERIB and VINO IENFD and all PK samples are pending. Overall, data suggest more severe deficits with PAC treatment and a longer recovery. Updated results will be presented for each endpoint analysis.

Disclosures: **K.M. Wozniak:** None. **Y. Wu:** None. **Y. Liu:** None. **R. Weinberg:** None. **V. Carozzi:** None. **G. Fumagalli:** None. **P. Alberti:** None. **B. Cook:** None. **S. Benbow:** None. **B.A. Littlefield:** A. Employment/Salary (full or part-time): Eisai Inc. **K. Nomoto:** A. Employment/Salary (full or part-time): Eisai Inc. **L. Wilson:** None. **M.A. Jordan:** None. **S. Feinstein:** None. **S. Eckley:** A. Employment/Salary (full or part-time): Eisai Inc. **C. DeJardins:** A. Employment/Salary (full or part-time): Eisai Inc. **G. Cavaletti:** None. **J. Mankowski:** None. **M. Polydefkis:** None. **B.S. Slusher:** 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; Eisai Inc.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.02/II4

Topic: D.01. Sensory Disorders

Title: PGN-503, a herpes simplex virus based vector expressing neurotrophin-3 for the prevention and treatment of chemotherapy induced peripheral neuropathy

Authors: ***J. R. GOSS**, D. KRISKY, K. BOUCH, M. O'MALLEY, S. COGHLAN, J. WECHUCK;
Periphagen Inc, Pittsburgh, PA

Abstract: Chemotherapy induced peripheral neuropathy (CIPN) is devastating constellation of symptoms that arise as a complication in patients receiving chemotherapy for cancer. It presents as a stocking and glove neuropathy characterized by numbness, paresthesia, and occasionally burning pain in the feet and hands. It can develop in up to 90% of all patients receiving chemotherapy, depending on the dose and type of drug. The development of CIPN may result in dose reduction of the chemotherapy agent, a switch to less efficacious agent, or even cessation of all chemotherapy treatment. Currently there are no approved therapies for the treatment of CIPN. PGN-503 is a new investigational drug designed to prevent and/or treat CIPN. It is a herpes simplex virus type 1 based vector expressing human neurotrophin-3 (NT-3). Following an intradermal injection, PGN-503 is taken up by sensory nerve terminals, transported back to the cell body, and directs the expression of NT-3. NT-3 is a well-studied neurotrophic factor

necessary for the growth and maintenance of sensory neurons. Neurotrophic factors, including NT-3, have been shown to be efficacious in the treatment of drug-induced neuropathies and the neuropathy associated with diabetes, however, attempts to use recombinant human trophic factor peptides in patients has proven difficult, likely due to the inability to deliver a sufficient amount at the site of the peripheral sensory nerves without adverse systemic side effects. In order to overcome this barrier, we have chosen to use a herpes simplex virus (HSV) vector to direct the expression of NT-3 directly in the peripheral sensory nerves at risk of developing neuropathy. In a series of preclinical studies, we examined the efficacy of PGN-503 in mouse models of CIPN induced by paclitaxel, oxaliplatin, and bortezomib. In all models a single injection of PGN-503 into the plantar surface of the hind paws 3 days prior to chemotherapy dosing, was sufficient to protect the mice from developing CIPN as assessed by electrophysiological analysis.

Disclosures: J.R. Goss: A. Employment/Salary (full or part-time): Periphagen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Periphagen. **D. Krisky:** A. Employment/Salary (full or part-time): Periphagen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Periphagen. **K. Bouch:** A. Employment/Salary (full or part-time): Periphagen. **M. O'Malley:** A. Employment/Salary (full or part-time): Periphagen. **S. Coghlan:** A. Employment/Salary (full or part-time): Periphagen. **J. Wechuck:** A. Employment/Salary (full or part-time): Periphagen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Periphagen.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.03/II5

Topic: D.02. Somatosensation: Pain

Title: Prevention of oxaliplatin-induced neurotoxicity involving decrease in peripheral blood flow by oral administration of goshajinkigan

Authors: T. KONO[1,2], *Y. OMIYA[3], H. SEKINE[3], M. YAMAMOTO[3], K. MIYANO[4], Y. KASE[3], Y. UEZONO[4];
[1]Fac. of Pharmaceut. Sci., Hokkaido Univ., Hokkaido, Japan; [2]Ctr. for Clin. and Biomed. Res., Sapporo Higashi Tokushukai Hosp., Hokkaido, Japan; [3]Tsumura Res. Labs., Tsumura & Co., Ibaraki, Japan; [4]Div. of Cancer Pathophysiology, Natl. Cancer Ctr. Res. Inst., Tokyo, Japan

Abstract: We have developed a useful rat model for the elucidation of oxaliplatin-induced chronic neuropathy and investigated the action of Goshajinkigan (GJG), a traditional Japanese herbal medicine. GJG has been reported to ameliorate oxaliplatin-induced neuropathy in a placebo-controlled double-blind randomized phase II study in Japan and is also refereed in chemotherapy-induced neurotoxicity guidelines (American Society of Clinical Oncology). A previous report observed that peripheral blood flow (PBF) was altered in oxaliplatin-treated animals, which might correlate with neurotoxicity. GJG and its components have been reported to show vasodilatation in several models partly via increasing nitric oxide production. Therefore, we investigated the efficacy of GJG on oxaliplatin-induced decrease of PBF. The effects of GJG were examined in rat following treatment with oxaliplatin (4 mg/kg, i.p.) twice weekly for 8 wks and with oral administration of GJG five times a week for 8 wks. PBF was measured using a two-dimensional laser speckle Blood Flow Imager. Rats were anesthetized. Body temperature was maintained at 37.5 ±0.5°C using a heating pad. Blood pressure and heart rate were concurrently measured by a tail-cuff apparatus. PBF in the head, ears, forelimbs, hindlimbs and tail of rats was measured at four defined time points (day 3, 10, 26 and 54). Real image and color-coded images were obtained. PBF value is expressed as mean blood flow. The ratio of blood flow rate of treated animals to naïve animals was calculated (%). Regional PBF, except for that in the ears and tail, decreased from 10 days to 4 wks after treatment with oxaliplatin, but then increased back toward normal values after 8 wks. Mean blood flow in the fore- and hindlimbs was significantly decreased at 4 wks. Reduction of mean blood flow in the left hindlimb was significantly inhibited in rats administered GJG at 4 wks after oxaliplatin treatment. No change in blood flow at each site was observed at 8 wks after oxaliplatin treatment. GJG did not affect either systemic blood pressure or heart rate in oxaliplatin-treated rats. Our study suggests that GJG prevents oxaliplatin-induced decrease of PBF which can be a crucial mechanism for preventing oxaliplatin-induced neuropathy.

Disclosures: **T. Kono:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Tsumura&Co. **Y. Omiya:** A. Employment/Salary (full or part-time): Tsumura&Co. **H. Sekine:** A. Employment/Salary (full or part-time): Tsumura&Co. **M. Yamamoto:** A. Employment/Salary (full or part-time): Tsumura&Co. **K. Miyano:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Tsumura&Co. **Y. Kase:** A. Employment/Salary (full or part-time): Tsumura&Co. **Y. Uezono:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Tsumura&Co..

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.04/II6

Topic: D.02. Somatosensation: Pain

Title: TRPM8-mediated response to cold in human DRG neurons and its modulation by the chemotherapy agent oxaliplatin

Authors: *A. GHETTI[1], J. ZHANG[1], Y. MIRON[1], J. STRETTON[2], K. MORRISON[2], P. MURDOCK[2], K. PAGE[2], P. MILLER[1];
[1]Anabios Corp., San Diego, CA; [2]Asterand Biosci., Royston, United Kingdom

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN), is a serious dose-limiting adverse effect induced by several chemotherapy agents and can lead to dose reduction or discontinuation of therapy, with consequent negative impact on cancer patients' survival. Manifestations of CIPN include: mechanical and cold allodynia, burning pain and tingling numbness. Following the cessation of chemotherapy, gradual recovery from peripheral nerve damage occurs in most patients but, in a significant number of cases, pain may persist. Cold allodynia is a very common instance with CIPN and growing efforts have been focused on the identification and development of pharmacological agents that can treat cold-related pain in CIPN patients. The mediators of cold pain sensation in human remain poorly characterized, although several ion channel candidates have been proposed. Transient Receptor Potential Cation Channel, Subfamily M, Member 8 (TRPM8) is considered a prime candidate for the transduction of low temperature-induced pain. For this reason, it is a potentially valuable target for the development of novel agents with the potential to treat cold allodynia. We have investigated the expression of TRPM8 in human dorsal root ganglion (DRG) neurons as well as in a variety of other tissues. Expression of TRPM8 mRNA was detectable by qRT-PCR (TaqMan®) not only in the DRG neurons, but also in several other tissues, including prostate, testis and liver. By employing automated branched-DNA *in situ* hybridization and automated immunohistochemistry on sections of fixed human DRG, we were able to localize TRPM8 expression to both large as well as smaller diameter DRG neuron cell bodies. We then conducted functional studies aimed at measuring the amplitude and kinetics of cold-induced responses in human DRG neurons in culture. In these preparations we recorded a rapid and reversible increase in intracellular calcium, following a rapid cooling to temperatures between 8°C and 12°C. Pre-treatment with a TRPM8 blocker resulted in marked, but incomplete, inhibition of the cold-induced responses. Pre-treatment of the cells with the chemotherapy drug oxaliplatin potentiated the response to rapid cooling and reduced the rate of desensitization. The increased duration of the cold-induced response in human DRG neurons, may contribute to the mechanism underlying CIPN-related

cold allodynia and may provide a useful *ex vivo* assay for identifying novel therapeutic agents for treating this condition.

Disclosures: **A. Ghetti:** A. Employment/Salary (full or part-time): AnaBios Corporation, Asterand. **J. Zhang:** A. Employment/Salary (full or part-time): AnaBios Corp. **Y. Miron:** A. Employment/Salary (full or part-time): AnaBios Corp. **J. Stretton:** A. Employment/Salary (full or part-time): Asterand Biosciences. **K. Morrison:** A. Employment/Salary (full or part-time): Asterand Biosciences. **P. Murdock:** A. Employment/Salary (full or part-time): Asterand Biosciences. **K. Page:** A. Employment/Salary (full or part-time): Asterand Biosciences. **P. Miller:** A. Employment/Salary (full or part-time): AnaBios Corp..

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.05/II7

Topic: D.01. Sensory Disorders

Title: Synergistic interaction of morphine plus clonidine in cisplatin-induced neuropathic pain in rat

Authors: ***A. ZUÑIGA**[1,2], J. REYES-GARCÍA[2], F. FLORES-MURRIETA[2], H. ROCHA-GONZÁLEZ[2];
[1]INER, MEXICO DF, Mexico; [2]Escuela Superior de Medicina IPN, ciudad de México, Mexico

Abstract: Clinical use of anti-neoplastic drugs is associated with the development of numerous adverse effects that many patients find intolerable, including peripheral neuropathy. Cisplatin, an effective anti-neoplastic agent in the treatment of solid tumors, produces a dose-limiting painful peripheral neurophathy in a clinically significant number of cancer patients. The aim of the present study was to investigate the possible synergistic interaction of systemic administration of morphine and clonidine either alone or in combination in an animal model of neuropathic pain evoked by cisplatin. Mechanical allodynia was measured by using von Frey filaments in the hindpaw. Male Wistar rats, weighting 160-180 g, were treated with cisplatin administered intraperitoneally three times a week (Monday, Wednesday, and Friday) at a dose of 0.1 mg/ 100 g weight. Morphine (0.1, 0.3, 3 and 5.6 mg/kg), clonidine (0.03, 0.3, 563 and 30 μg/kg), or equieffective doses of the combination in a ratio 1:1 were administered to obtain the 40% experimental effective doses (ED_{40}) for each drug and for the combination. Isobolographic analysis was performed to examine the interaction. Morphine (ED_{40} = 3.2 +/- 0.7 mg/kg), clonidine (ED_{40} = 0.88 +/- 3.5 μg/kg), and fixed-dose ratio morphine-clonidine combinations showed dose-dependently anti-allodinical effect. Theoretical ED_{40} value for the combination

estimated from the isobologram was 2.05 +/- 0.32 mg/kg, whereas that experimental ED_{40} value was 0.80 +/-0.02 mg/kg. Results indicate that i.p. administration of morphine plus clonidine can interact synergistically in the neuropathic pain model and suggest the use of this combination to relieve pain evoked by anti-neoplastic agents in humans.

Disclosures: A. Zuñiga: None. **J. Reyes-García:** None. **F. Flores-Murrieta:** None. **H. Rocha-González:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

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Program#/Poster#: 426.06/II8

Topic: D.02. Somatosensation: Pain

Support: NIH CA111891

NIH CA165202

The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Diseases Research

Title: The therapeutic effect of Rho kinase inhibitor Y-27632 on protection from chemotherapy-induced peripheral neuropathy in a tumor-bearing mouse model

Authors: *Y. ZHU[1], G. A. HOWARD, IV[2], K. PITTMAN[2], C. BOYKIN[1], K. VERBANAC[2], Q. LU[1];
[1]Anat. and Cell Biol., [2]Surgery, Brody Sch. Of Med., Greenville, NC

Abstract: Cisplatin often causes loss of touch sensitivity in the hands and feet of cancer patients as well as tingling, numbness, and a shooting or burning pain; these clinical symptoms are referred to as chemotherapy-induced peripheral neuropathy (CIPN). CIPN frequently results in a reduction or cessation of chemotherapy, and there is currently no effective intervention or prevention for CIPN. Therefore, it is important to understand the mechanism of CIPN pathogenesis and determine associated signaling pathways to identify potential therapeutic targets. Previously, we created a CIPN mouse model in 3 month old C57/BL6 mice by injections of 6μg/g cisplatin every 14 days. Our data indicated that the RhoA signaling pathway was responsible for attenuating CIPN since the preserved touch sensitivity was achieved in cisplatin-treated mice by injecting LM11A-31, a p75 neurotrophin receptor ligand mimetic that serves as an upstream inhibitor of RhoA signaling pathway. In order to fully capture the clinical situation, we extend this approach to CIPN in a syngeneic murine Lewis Lung Carcinoma (LLCab) model

in which mice were concurrently treated with weekly injections of cisplatin and a RhoA pathway inhibitor. In this tumor-bearing CIPN mouse model, we determined the therapeutic effectiveness of Y-27632 that selectively inhibits ROCK, a downstream effector of the RhoA signaling pathway, in peripheral neuroprotection. Von Frey filaments analysis of hind paw touch sensitivity indicated that Y-27632 treatment protected tumor-bearing mice from cisplatin-induced reduction of touch sensation. Furthermore, immunohistochemical analysis of cutaneous nerve fibers in foot pad tissue, which was acquired from the corresponding hind paw, demonstrated that the cisplatin-induced decrease in touch sensory associated-cutaneous nerve fibers could be alleviated by the concurrent treatment with Y-27632. Therefore, Rho kinase inhibitor Y-27632 can potentially protect peripheral nerve function in the tumor-bearing CIPN mouse model.

Disclosures: Y. Zhu: None. **G.A. Howard:** None. **K. Pittman:** None. **C. Boykin:** None. **K. Verbanac:** None. **Q. Lu:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.07/II9

Topic: D.02. Somatosensation: Pain

Support: NS046606

H.E.B.Professorship in Cancer Research

CA200263

Title: The role of TLR4 signaling pathway in oxaliplatin-induced peripheral neuropathy in rat

Authors: *P. M. DOUGHERTY[1], A. ILLIAS[2], Y. LI[2], H. ZHANG[2], K.-J. YU[2], J. F. VELASQUEZ[3], J. P. CATA[2];
[1]Pain Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]MD Anderson, Houston, TX; [3]MD Anderson.org, Houston, TX

Abstract: Many efforts have been made to understand the mechanism of Chemotherapy-Induced Peripheral Neuropathy (CIPN) in order to treat or prevent this common complication of cancer treatment, which sometimes persists even after the patient is cured from cancer. Accumulating evidence shows that TLR4 signaling contributes to paclitaxel-induced peripheral neuropathy. In the present study, we test the hypothesis that TLR4 also plays a crucial role in peripheral neuropathy caused by platin agents, Oxaliplatin in particular. Peripheral neuropathy was induced

in a group of Sprague-Dawley rats treated with oxaliplatin. Mechanical hypersensitivity test (von Frey's), biochemical analysis (Western blot), and immunohistochemistry staining results from rats treated with oxaliplatin were compared to a group of control rats and another group of rats who received intrathecal injections of TLR4 antagonist LPS-RS along with intraperitoneal oxaliplatin. Oxaliplatin treated rats expressed decrease in paw withdrawal threshold detected one hour post oxaliplatin injection and continued as long as our experiment was conducted, for 35 days after the first injection, whereas blocking of TLR4 with LPS-RS resulted in a behavior phenotype closer to the control group. Western blot show that TLR4 protein along with its downstream signaling molecule- myeloid differentiation primary response gene 88 (MyD88)- were increased in dorsal root ganglion (DRG) since 7 days after oxaliplatin injection and continued throughout day 35. Furthermore, Immunohistochemistry staining displayed increases in expression of glial fibrillary acidic protein (GFAP) positive cells in dorsal horn of oxaliplatin treated rats with colocalization of TLR4 on these cells. In the same group, TLR4 was also increased in calcitonin gene-related peptide (CGRP) and isolectin B4 (IB4) positive DRG cells. Co-administration of LPS-RS prevented excessive expression of TLR4 as detected by both immunohistochemistry and western blot analysis. Therefore, Blockade of TLR4 might prevent or attenuate pain caused by chemotherapy agents.

Disclosures: P.M. Dougherty: None. **A. Illias:** None. **Y. Li:** None. **H. Zhang:** None. **K. Yu:** None. **J.F. Velasquez:** None. **J.P. Cata:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.08/II10

Topic: D.02. Somatosensation: Pain

Support: R01NS87988

R01DE22743

Title: Neuroprotectin D1 protects the chemotherapy-induced neuropathy by modulating the function of macrophages

Authors: *S. BANG, Z.-Z. XU, R.-R. JI;
Dept. of Anesthesiol. and Neurobio., Pain Res. Div. ,Department of Anesthesiol. and Neurobiology, Duke Univ., Durham, NC

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a major side-effect of cancer treatment. Neuroprotectin D1 (NPD1) is derived from omega-3 unsaturated fatty acids and has potent anti-inflammatory and pro-resolution actions. Our previous study showed that NPD1 prevented the nerve injury-induced neuropathic pain by regulating glial activation and neuroinflammation in the spinal cord. In the present study, we examined whether NPD1 would modulate macrophage function following paclitaxel-induced CIPN. Systematic injection of NPD1 effectively prevented paclitaxel-induced mechanical allodynia in mice. Paclitaxel also induced marked infiltration of macrophages to dorsal root ganglia (DRG), but this infiltration was blocked by the NPD1 treatment. NPD1 also reduced the production of proinflammatory cytokines in macrophages from the paclitaxel injected mice. In the primary cultures of macrophages, NPD1 further enhanced the phagocytosis activity and reduced the production of proinflammatory cytokines. Intraplantar injection of the paclitaxel-treated but not control macrophages induced mechanical allodynia, but this allodynia was compromised by pretreatment of paclitaxel-stimulated macrophages with NPD1. These results suggest that NPD1 might protect paclitaxel-induced neuropathic pain through regulation of macrophage function.
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Disclosures: **S. Bang:** None. **Z. Xu:** None. **R. Ji:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

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Program#/Poster#: 426.09/II11

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS046606

H.E.B. Professorship in Cancer Research

Title: Contribution of voltage-gated sodium channel 1.7 in rat in paclitaxel induced peripheral pain

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Abstract: Paclitaxel chemotherapy induced peripheral neuropathy (CIPN) is a common side effect experienced by patients in chemotherapy. Although the mechanisms remain little understood several lines of evidence indicate that CIPN is associated with hyperexcitability of peripheral and central neurons. Here the role of changes in expression and function of the voltage-gated sodium channel Nav1.7 in DRG and spinal cord in CIPN was tested in rats. Double immunofluorescence staining showed that Nav1.7 was up-regulated in small DRG neurons especially those also expressing CGRP. Meanwhile and in contrast Nav1.7 was down regulated in spinal cord, in particular being reduced in GAD65 and GAD67 positive spinal neurons. Whole-cell patch clamp recordings in DRG neurons and lamina II spinal cord neuron revealed that paclitaxel (1)induced a prominent enhancement of I_{NaT} and (2)increased ramp currents, the persistent sodium current (I_{NaP}), in response to slow depolarization and (3)decreased the frequency of sIPSC in spinal cord dorsal horn neurons. The reduction in spinal neuron sIPSC was rescued by perfusion with the Nav1.7 blocker ProTx II. Intrathecal injection of ProTx II also significantly attenuated the behavioral signs of CIPN. This study suggests that Nav1.7 may provide a potential new target for the treatment of paclitaxel induced peripheral neuropathic pain.

Disclosures: Y. Li: None. **D.D. Edwards:** None. **R.M. Cassidy:** None. **D.S. Harrison:** None. **A.K. Kosturakis:** None. **H. Zhang:** None. **P.M. Dougherty:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.10/II12

Topic: D.02. Somatosensation: Pain

Support: GAUK 138215

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RVO67985823

Title: Paclitaxel application leads to long-term changes of presynaptic TRPV1 receptors function in spinal cord dorsal horn

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Abstract: Antineoplastic drug Paclitaxel (PTX) is the frontline agent widely used in clinical practice for the treatment of multiple solid tumors, such as breast, ovarian and lung cancer. However, the major dose- and treatment-limiting factor of PTX therapy is development of Chemotherapy-Induced Peripheral Neuropathy (CIPN). We have shown previously (Li et al., J. Neurosci., 35:13487-13500, 2015) that acute application of PTX (50 nM) modulated frequency of mEPSC via TLR4 and TRPV1 receptors activation. PTX also significantly reduced tachyphylaxis present after repeated capsaicin application. In extension of these findings we have studied the effect of PTX treatment on the capsaicin response tachyphylaxis and behavioral changes at different time points. Whole-cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSC) were made from superficial dorsal horn neurons in acute spinal cord slices prepared from adult male mice C57BL/6. CIPN was induced by single dose application of PTX (Mylan, 8 mg/kg; *i.p.*). Von Frey filament measurements were used for behavioral testing to evaluate the presence of mechanical allodynia in the model. In naïve animals mEPSCs frequency evoked by second capsaicin (0.2 μM) application was dramatically reduced to 33 % of the first one. After acute PTX treatment the second response was 91 % of the first one. Our latest data show that PTX significantly changed the second capsaicin response also after incubation in PTX (50 nM) for ~2 hours (80 %) and 1 (72 %) and 8 days (83 %) after single systemic PTX (8 mg/kg; *i.p.*) treatment. The systemic PTX treatment also significantly increased the basal frequency of mEPSC (2.5 Hz and 3.2 Hz vs. 0.9 Hz in controls) and enhanced responses to capsaicin (22.2 Hz and 26.2 Hz vs. 7.8 Hz in controls) at 1 and 8 days after the PTX treatment. Further information about the intracellular signaling pathways involved in the signaling between the TLR4 and TRPV1 receptors after the *in vitro* and *in vivo* PTX treatment will be also presented on-site. Our results suggest that functional interaction between TLR4 and TRPV1 receptors and modulation of TRPV1 receptors properties in the spinal cord dorsal horn may play an important role in the development painful states after PTX treatment. Targeting these receptors may represent a viable option for possible analgesic treatment.

Disclosures: **P. Adamek:** None. **J. Palecek:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.11/II13

Topic: D.02. Somatosensation: Pain

Support: Intradepartmental fund, The University of Texas MD Anderson Cancer Center

Title: HKP-16 ameliorates chemotherapy-induced neuropathic pain in rats by oral administration

Authors: *H. KIM, S.-H. HWANG, S. ABDI;
Dept. of Pain Med., MD Anderson Cancer Ctr., Houston, TX

Abstract: Chemotherapy agents including taxanes, vinca alkaloids, platinum complexes, and proteasome inhibitor produce peripheral neuropathic pain which is a dose-limiting side effect. Paclitaxel (PAC) is the first choice for breast cancer and produced neuropathic pain. Commonly used pain medications such as opioids, anticonvulsants, antidepressants, and selective serotonin norepinephrine reuptake inhibitors have only modest analgesic effects at best for chemotherapy-induced neuropathic pain. Therefore, we need to develop new drugs. The purpose of this study was to determine the analgesic effects of HKP-16 as a new drug candidate on PAC-induced neuropathic pain in rats. PAC-induced neuropathic pain model was produced by intraperitoneal injections of PAC (2 mg/kg; total doses of 8 mg/kg) on days 0, 2, 4, and 6 in adult male Sprague-Dawley rats. The behavioral tests for mechanical hyperalgesia were foot withdrawal thresholds to von Frey filaments. After the rats developed pain behavior, HKP-16 was orally administered in the suspension in 0.5% methylcellulose in water. The single oral administration of HKP-16 reduced PAC-induced pain behaviors in rats at the doses of 10, 30, and 100 mg/kg. Especially, the 100 mg/kg returned the pain up to no-pain condition. In addition, repeated oral administrations of HKP-16 (twice daily for 4 days at the dose of 30 mg/kg) significantly reduced PAC-induced pain behaviors for 5 days. The single and repeated administrations of HKP-16 did not produce sedation as a side effects. The result indicates that HKP-16 ameliorates PAC-induced neuropathic pain in rats by oral administration. We conclude that HKP-16 may be a potent new drug candidate for chemotherapy-induced chronic neuropathic pain in cancer patients and survivors.

Disclosures: H. Kim: None. **S. Hwang:** None. **S. Abdi:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.12/II14

Topic: D.02. Somatosensation: Pain

Support: Intradepartmental fund, The University of Texas MD Anderson Cancer Center

Title: Pentoxifylline decreases inflammatory cytokines in the dorsal root ganglia in chemotherapy-induced neuropathic pain in rats

Authors: *S.-H. H. KIM, H. KIM, S. ABDI;
Pain Med. - Res., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Many chemotherapy agents such as taxanes, vinca alkaloids, and platinum complexes produce peripheral neuropathic pain, which is a dose-limiting side effect. We previously reported that pentoxifylline (PTX) ameliorates paclitaxel (PAC)-induced neuropathic pain in rats. The purpose of this study was to investigate the effect of PTX on PAC-induced inflammatory cytokines in dorsal root ganglia (DRGs). PAC (2 mg/kg on days 0, 2, 4, 6) or vehicle (4% dimethyl sulfoxide and 4% Tween 80 in saline) was intraperitoneally injected in adult male Sprague-Dawley rats. PTX was intraperitoneally infusion for 7 days starting on day 14 after the first PAC injection and the DRG was dissected on day 20. For western blot, the L1-6 DRGs were dissected, homogenized in RIPA lysis buffer, separated in SDS polyacrylamide gel and then transferred to polyvinylidene fluoride membrane. For detection, blot was incubated with the primary antibody to phosphorylated NFκB (p-NFκB), IL-1β, TNF-α, and GAPDH, respectively and then incubated with the horseradish peroxidase-conjugated secondary antibody. The immunoblots were detected by a chemiluminescence detection system and normalized to GAPDH. For immunohistochemistry, the L5 DRG was dissected, post fixed, cryoprotected in 30% sucrose solution, cryosectioned, and mounted on slides. The section was incubated with primary antibodies followed by secondary antibodies conjugated with either Alexa Fluor 568 or Alexa Fluor 488. The primary antibodies used anti-NeuN, anti-glial fibrillary acidic protein (anti-GFAP), and anti-IL-1β. PAC significantly increased the levels of p-NFκB, TNF-α and IL-1β in the DRGs. Further, PAC-induced increase in p-NFκB, TNF-α, and IL-1β protein levels in the DRGs were decreased by PTX treatment. In addition, IL-1β was expressed in the L5 DRG in PAC-injected rats and IL-1β was co-expressed in NeuN-positive neurons and GFAP-positive satellite cells in the L5 DRG. This result indicates that PAC increases the production of phosphorylated NFκB, TNF-α, and IL-1β in the DRGs and inhibition of PAC-induced inflammatory cytokines level in the DRGs may be the mechanisms underlying the analgesic effect of PTX for chemotherapy-induced neuropathic pain in rats.

Disclosures: S.H. Kim: None. **H. Kim:** None. **S. Abdi:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.13/II15

Topic: D.02. Somatosensation: Pain

Support: NIH 1 P20 GM103643-01A1

Title: The Neuronal calcium sensor-1 knockout mouse model and its utility in better understanding chemotherapy-induced neuropathic pain

Authors: E. M. EDWARDS[1], A. FERRAR[1], D. GIUVELIS[1], K. LINDROS[1], I. M. BERGQUIST[1], O. PONGS[3], E. KAFTAN[4], B. EHRLICH[4], *E. J. BILSKY[2];
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Abstract: The neuronal calcium sensor gene family encodes calcium-binding proteins (including NCS-1) that are predominantly expressed in neurons and regulate G protein-coupled receptor phosphorylation in a calcium-dependent manner. NCS-1 regulates a number of intracellular functions including synaptic plasticity and transmission. We have previously shown that NCS-1 is critical to the cellular pathway responsible for paclitaxel-induced peripheral neuropathy. Prolonged treatment with paclitaxel induced degradation of NCS-1 and compromised intracellular calcium signaling. An NCS-1 knockout mouse (NCS-1 KO) has been developed to further explore this mechanistically and we are in the process of doing a comprehensive phenotyping, as well as using the model to assess the role of NCS-1 in development of chronic pain. Adult male and female WT and NCS-1 KO mice were run through a battery of standardized sensory, nociceptive, motor and other behavioral assays to determine the KO phenotype in preparation for the more advanced studies that are examining and comparing nociceptive responses to paclitaxel and other injury-induced nociceptive models. In open field locomotor studies, the WT and NCS-1 KO mice were indistinguishable from each other. Similar results were found with rotarod (coordination) and grip strength measures. Trait anxiety measures were normal between the two groups. Responses to acute thermal stimuli (hot and cold) were generally similar across a range of stimuli intensities, as were tactile thresholds. A small difference was observed in response to plantar incision hyperalgesia (thermal) with NCS-1 KO mice showing a reduced response. In female mice, initial results indicate that paclitaxel produced a similar degree of hypersensitivity in WT and KO mice (male mice are currently being tested). The NCS-1 KO mouse displayed a near normal behavioral phenotype compared to corresponding WT controls and should be a useful tool in further exploring the pathophysiology of chemotherapy induced peripheral neuropathies.

Disclosures: **E.M. Edwards:** None. **A. Ferrar:** None. **D. Giuvelis:** None. **K. Lindros:** None. **I.M. Bergquist:** None. **O. Pongs:** None. **E. Kaftan:** None. **B. Ehrlich:** None. **E.J. Bilsky:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.14/II16

Topic: D.03. Somatosensation: Touch

Support: NS-057228

Title: Oxaliplatin impairs mechano-sensory encoding by slowly adapting cutaneous afferents

Authors: J. A. VINCENT[1], P. NARDELLI[2], *T. C. COPE[2];
[1]Neuroscience, Cell Biology, and Physiol., Wright State Univ., Dayton, OH; [2]Applied Physiol. and Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Chronic oxaliplatin (OX) neurotoxicity causes debilitating effects including sensory ataxia and paraesthesia. Our *in vivo* studies (Bullinger et al 2011, Vincent et al 2015) of rats weeks after a full treatment course of OX demonstrate that muscle proprioceptors lose their ability to sustain repetitive firing during static muscle stretch. Given this finding and the known effects of OX on cutaneous sensation (Reddy et al 2016), we hypothesize that OX also abbreviates sustained firing to in response to static tactile stimulation of low threshold mechanoreceptors (LTMRs) in the skin. We began testing this hypothesis by measuring LTMR firing responses in 2 rats following OX treatment after first establishing stable characterization of responses in 10 untreated control rats. Firing was measured in terminal experiments on adult female Wistar rats anesthetized with isoflurane. Action potentials were recorded intra-axonally in dorsal roots from single LTMRs responding to tactile stimulation of glabrous skin on the plantar surface of the foot. LTMRs characterized as Aβ based on conduction delay and threshold were selected for detailed study. Receptors were classified as rapidly adapting (RA) when they adapted and ceased firing during the first 3 seconds of sustained skin indentation, whereas slowly adapting (SA) receptors maintained repetitive firing during sustained skin displacement for 10 seconds and up to 60 secs. Further differentiation of RA1 or 2 vs. SA1 or 2 afferents was based on their vibration sensitivity, and the regularity of firing during sustained skin indentation, respectively. Similar to previous studies of rodent LTMRs, RA afferents ceased firing within 3-5 second of sustained indentation; differentiation of RA2 afferents was based on their exquisite sensitivity to high frequency vibration. SA afferents maintained discharge during sustained displacement, SA1 afferent discharge was highly variable, whereas SA2 afferents showed a

highly regular discharge (Leem et al 1993, Woodbury 2007, Wellnitz 2010, Lesnoak 2014). By comparison, preliminary findings for rats following chronic OX treatment indicated that SA2 afferents had difficulty maintaining repetitive firing during sustained skin displacement, SA1 afferents appeared unaffected as did RA afferents. These early results suggest that chronic OX toxicity might selectively impair signaling of static mechanical stimuli for cutaneous afferents as it does for muscle afferents. These results warrant further study as they may help to explain altered cutaneous sensation experienced by patients treated with OX.

Disclosures: **J.A. Vincent:** None. **P. Nardelli:** None. **T.C. Cope:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

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Program#/Poster#: 426.15/II17

Topic: D.02. Somatosensation: Pain

Title: Fy-504, a potent and selective nav1.8 antagonist, blocked chemotherapy-induced peripheral neuropathy (cipn)

Authors: *P. CHO;
Physiol. Lab., Seoul, Korea, Republic of

Abstract: The NaV1.8 sodium channel, encoded by gene SCN10A, was initially termed sensory neuron-specific (SNS) due to prominent expression in primary sensory neurons including dorsal root ganglion (DRG) neurons. This Na(v)1.8 sodium channel has tetrodotoxin-resistant property, activation of which contributes to action potential generation in DRG neurons. Recent genetic and pharmacological evidence indicates that activation of Na(v)1.8 channels contributes to chronic pain, such as inflammatory and neuropathic pain. Here we propose a new selective and potent Na(v)1.8 antagonist (FY-504), which is a modified natural compound from Asian sweetleaf. FY-504 potently blocked tetrodotoxin(TTX)-resistant sodium currents (IC(50)=10pM) from small diameter mouse DRG neurons in a voltage-dependent fashion, but not TTX-sensitive voltage-gated sodium channels. In addition, FY-504 potently blocked recombinant human Na(v)1.8 channels in a dose dependent manner(IC50 ~10 pM) and completely blocked Na(v)1.8 at 100 pM, but did not affect the sodium current of HEK cell with Na(v)1.5 or 1.9. Next, we investigated whether FY-504 effectively suppresses chemotherapy-induced peripheral neuropathy (CIPN). CIPN is a major dose-limiting toxicity of potentially curative cancer therapy regimens. Chemotherapy agents, such as cisplatin and vincristin, have a broad spectrum of activity against tumors, but they induce peripheral sensory neuropathy and there is currently no accepted proven prevention or therapy of CIPN. In CIPN model, protein expression of Na(v)1.8

in DRG and spinal cord increased and FY-504 alleviated painful behavior of CIPN model(vincristin-induced peripheral neuropathy). Thus, FY-504 may also be a promising novel candidate for the prevention and treatment of CIPN.

Disclosures: P. Cho: None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.16/JJ1

Topic: D.01. Sensory Disorders

Title: The role of fscns1 in peripheral nerve regeneration

Authors: *T. OMURA[1], D. XU[1], T. BANNO[1], A. OKAMOTO[1], K. OMURA[2], Y. MATSUYAMA[1];
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Abstract: We screened nine genetically diverse inbred mouse strains including seven founder lines of the collaborative cross in attempt to discover novel genes and pathways to promote peripheral nerve regeneration. Naïve and conditioned L4 and 5 adult dorsal root ganglion (DRG) explants from A/J, C3H/HeJ, C57BL/6J, DBA/2J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, and WSB/EiJ strains were grown and assayed on Matrigel, mimicking peripheral nervous system (PNS) environment for their ability to extend axons. *In vitro* analysis for total longest axonal length revealed DBA as the most regenerating strain in the PNS environment. In vivo pinch test also showed 1.6 times more axonal growth with DBA in comparison with C57. Full-genome expression profiling of naïve and preconditioned DRGs across all strains revealed Taf7l and Fscn1 as the transcripts whose expressions most closely correlated with axonal growth on Matrigel. Taf7l is a member of the TATA binding protein associated factor 7 involved in forming transcription factor IID complex, and interestingly regulate Fscn1. Fscn1 is an actin filament binding protein which assembles actin filament binding and organizes actin cytoskeleton. Expression of Fscn1 mRNA in the DRG showed upregulation after peripheral nerve injury and in the conditioned primary cultured sensory neurons, increase in Fscn1 protein was observed in the regenerating axons. Our results indicate that Taf7l and Fscn1 could be playing an important role in peripheral nerve regeneration.

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Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

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Program#/Poster#: 426.17/JJ2

Topic: D.01. Sensory Disorders

Support: LLHF

Tau Consortium

Title: Axonal dysfunction in rab7-associated cmt2b peripheral sensory neuropathy

Authors: ***C. WU**[1], X. CHEN[2], X. ZHAO[2], W. YANG[2], C. JOLIVALT[2], L. BAO[3];
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Abstract: CMT2B is a rare but debilitating disease with axonopathy, i.e. axonal dysfunction and degeneration of the peripheral sensory neurons as a major clinical manifestation. Patients with this disease lose pain sensation and frequently need amputation. Genetic analyses have discovered missense autosomal dominant point-mutations at four residues (L129F, K157N, N161T/I, V162M) in Rab7 associated with the disease. Currently, the pathogenic mechanism is unknown, which has hindered the development of treatments. Thus far, studies using cell lines or Drosophila have led to two opposite conclusions: a “gain of toxicity” in mammalian cell lines versus a “loss of function” or haploinsufficiency in flies. They thus suggest two different therapeutic strategies: inhibiting activation of Rab7 for the “gain of toxicity” model and increasing expression or activation of Rab7 for the “halpoinsufficiency” model. Therefore, better models (rodent models, derived human neurons) are pivotal in resolving the differences in defining the disease mechanism(s) to discover critical pathway(s) towards therapeutic treatments. Based on human CMT2B-Rab7V162M patients10, we have generated a Rab7V162M knockin (KI) mouse model to better study the disease. Preliminary behavioral tests demonstrated that the KI Rab7V162M allele has an autosomal dominant effect and the mutant mice show significant deficits in peripheral sensory function. A thorough and careful study of the KI Rab7V162M mutant mice (heterozygotes: KI/+) and wildtype littermate controls (+/+) will yield important insight into the molecular and cellular mechanisms for CMT2B pathogenesis both in vivo and in vitro. We have also obtained human patient CMT2B-Rab7V162M fibroblasts for converting into human neurons1. The mouse model and patient-derived neurons will provide the best possible systems to study CMT2B.

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Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

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Topic: D.01. Sensory Disorders

Support: National Council for Scientific and Technological Development (CNPq),

Higher Education Personnel Improvement Coordination (CAPES)

Title: Oral administration of Compound A, which presents anti-inflammatory properties, ameliorates diabetic neuropathy

Authors: *F. H. P. MACEDO[1], R. D. AIRES[2], R. C. M. FERREIRA[3], D. P. D. MACHADO[3], T. R. L. ROMERO[3], J. H. LEAL-CARDOSO[4], J. S. CRUZ[2];
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Abstract: Diabetes Mellitus is a common condition that can lead to peripheral neuropathy, which cause painful symptoms in 16-34% of the diabetic patients. Recent studies have indicated that TNF-alpha might be directly correlated with the pain-related symptoms associated to diabetic neuropathy (DN). In an attempt to attenuate the increase in the TNF-alpha serum levels and the resulting DN development, we performed a daily gavage treatment (200 mg/kg) in streptozotocin-induced diabetic rats (65 mg/kg of streptozotocin via i.p. in 28 days old Wistar male rats) with our Compound A, which holds potent anti-inflammatory effects. Streptozotocin-induced diabetic rats (glycemic level > 300 mg/dl) were randomly separated in two groups, treated and untreated. The same was made for control animals. Rats from the groups control treated and diabetic treated received, daily, the Compound A for a period of 7 weeks, and then were euthanized for DRG dissection and neuron dissociation for total sodium current measurement by patch clamp technique. All protocols were approved by the Ethics Committee in Animal Experimentation (CEUA) from Universidade Federal de Minas Gerais (UFMG) - Protocol number 233/2013. Right before euthanasia, the diabetic rats presented increased in glycemic levels and the treatment with Compound A wasn´t able to reduce this glycemia in diabetic rats. In control rats this compound didn´t change the glycemic levels. Mechanical Threshold (g) were accessed by Randal-Selitto test, and before the diabetes induction, the mechanical threshold values were not different between groups. However, before euthanasia, diabetic rats showed lower mechanical threshold value when compared to control rats. The treatment with Compound A raised this threshold but it still was lower than control rats. In addition, the total sodium current density (pA/pF) in DRG neurons from diabetic rats was enhanced. Interesting, Compound A normalized this sodium current densities at control value.

TNF-alpha serum level measured just before the euthanasia was biggest in diabetic rats and the treatment with Compound A reduced this TNF-alpha concentration at same level to the control group. The decrease of the mechanical threshold associated with the increase of the total sodium current density in the diabetic group compared with the control group might be correlated with the elevation of the TNF-alpha serum levels in diabetic group rats. We conclude that the Compound A was able to attenuate the development of DN probably due to its capacity of preventing the elevation in TNF-alpha serum level.

Disclosures: F.H.P. Macedo: None. **R.D. Aires:** None. **R.C.M. Ferreira:** None. **D.P.D. Machado:** None. **T.R.L. Romero:** None. **J.H. Leal-Cardoso:** None. **J.S. Cruz:** None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.19/JJ4

Topic: B.06. Neurotransmitter Release

Support: RO1 NS075156

Title: Exosomes derived from schwann cells ameliorates periheral neuropathy in type II diabetic mice

Authors: *A. SZALAD[1], L. WANG[2], M. CHOPP[2], X. LU[2], L. JIA[2], M. LU[2], Y. ZHANG[2], R. ZHANG[2], X. LIU[2], Z. ZHANG[2];
[1]Neurol. Research, E&R Bld 3046, [2]Henry Ford Hosp., Detroit, MI

Abstract: Background: Peripheral neuropathy is one of the major common complications of diabetes. Exosomes, small microvesicles (30~100 nm) containing proteins, lipids and RNAs including microRNAs (miRNAs), mediate intercellular communication by transferring exosomal cargo between source and target cells. Myelin-forming Schwann cells (SCs) interact with axons and blood vessels to regulate peripheral nerve remodeling. Here, we investigated the effect of SC-derived exosomes on diabetic peripheral neuropathy. Methods and Results: Exosomes were isolated from supernatant of SCs cultured under exosome free medium. Male BKS.Cg-*m*+/+*Lepr*^{db}*/J* (db/db) mice at aged 20 weeks (n=10/group) were treated with SC-exosomes ($4x10^{10}$particles/mouse, i.v.) or saline bi-weekly for 8 consecutive weeks. Compared to diabetic mice treated with saline, diabetic mice treated with the exosomes exhibited significant ($p<0.05$) improvement of motor (MCV, 45.9±1.4 m/s vs. 34.3±0.9 m/s in saline) and sensory (SCV, 44.3±1.6 m/s vs. 35.0±1.5 m/s in saline) conduction velocity in the sciatic nerve**.** **H**istopathological analysis shows that the exosome treatment of diabetic mice significantly

($p<0.05$) increased sciatic nerve fiber diameter (9±0.8% vs. 7.7±1.0% in saline) and myelin thickness (1.9±0.3% vs 1.4±0.3% in saline), and robustly ($p<0.05$) reduced g-ratio (axon diameter/fiber diameter, 0.6±0.04% vs. 0.62±0.05% in saline). The exosome treatment also markedly ($p<0.05$) reduced thermal latency (8.0±0.7m/s vs. 11.2±0.7 m/s in saline) and increased of PGP9.5 positive sensory nerve fibers (14.3±0.9% vs. 10.7±0.7% in saline) in footpad epidermal tissue. Moreover, the exosome treatment substantially improved ($p<0.05$) regional blood flow (98±12% vs. 61±9% in saline) and the density of FITC-dextran perfused vessels (23.6±2% vs. 13.2±2% in saline) at the sciatic nerve of diabetic mice. Quantitative RT-PCR and Western blot analysis of sciatic nerve tissue showed that compared to non-diabetic mice, diabetes considerably ($p<0.05$) reduced miR-27a (0.3±0.04 vs. 1.0±0.1 in non-diabetic mice) and increased its target proteins SEMA6A (2.2±0.2 vs. 1.0±0.1 in non-diabetic mice), whereas the exosome treatment overcame the diabetic effect on miR27a (0.75±0.08 vs. 0.3±0.04 in saline) and SEMA6A (1.3±0.1 vs. 2.2±0.2 in saline). Conclusion: Treatment of diabetic mice with SC-exosomes remarkably improved sciatic nerve neurovascular function and ameliorated neurological functional outcome of peripheral neuropathy, suggesting a prominent therapeutic role for SC-exosomes in diabetic peripheral neuropathy.

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Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.20/JJ5

Topic: D.07. Vestibular System

Support: EC Grant 610454 EmBalance

Title: Sensory weighting in elderly people and neuropathy patients

Authors: *C. F. MAURER;
Neurozentrum, Freiburg, Germany

Abstract: INTRODUCTION: Postural control in elderly people as well as in neuropathy seems to be impaired by some similar factors. Changes in sensory systems include a reduced joint position sense at the ankle. Moreover, the motor system is affected in both conditions. However, few studies have systematically evaluated the role of sensory, motor, and central adaptation mechanisms for abnormal postural control in both, elderly and neuropathy. Using a feedback model-based approach, we aimed to identify, quantify, and compare these basic postural

mechanisms and, in addition, evaluate the effect of balance training on postural control in both conditions. METHODS: 17 elderly, 38 young people and 14 patients suffering from neuropathy were included. All patients underwent thorough clinical examination to exclude any additional deficit or neurological condition contributing to postural control. Balance control experiments were conducted using a continuous external perturbation, in this case, a support surface rotation, which followed a pseudo-random ternary sequence (PRTS). Patients had their eyes either closed or open. We applied perturbations with 0.5 and 1.0 degrees peak-to-peak amplitude. System identification and parameter estimation were used to estimate balance control model parameters describing the balance behaviour, including the sensory weights. RESULTS: Whereas neuropathy patients down-weight proprioceptive information, elderly people up-weight proprioception, and down-weight vestibular information. Balance training influenced the abnormal use of sensory information in both conditions, but in a diametrically opposed way. In elderly people, we identified, in addition, an increase in overall time delay challenging the feedback systems stability, and a decline in the amplitude of the motor feedback. These abnormalities were not found in neuropathy patients and were not influenced by balance training, either. DISCUSSION: The perturbation-based approach used here gives us more insight about postural control mechanisms of elderly people and neuropathy patients than measures of spontaneous sway do. In addition, it allows for a model-based interpretation of the experimental data and a precise identification of the therapeutic effects of balance training. The model parameters that were identified as critical parameters for the abnormal postural control in both conditions, were well correlated with clinical measures of balance which underlines their relevance for characterizing postural abnormalities.

Disclosures: C.F. Maurer: None.

Poster

426. Peripheral Neuropathy: Mechanisms and Interventions

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 426.21/JJ6

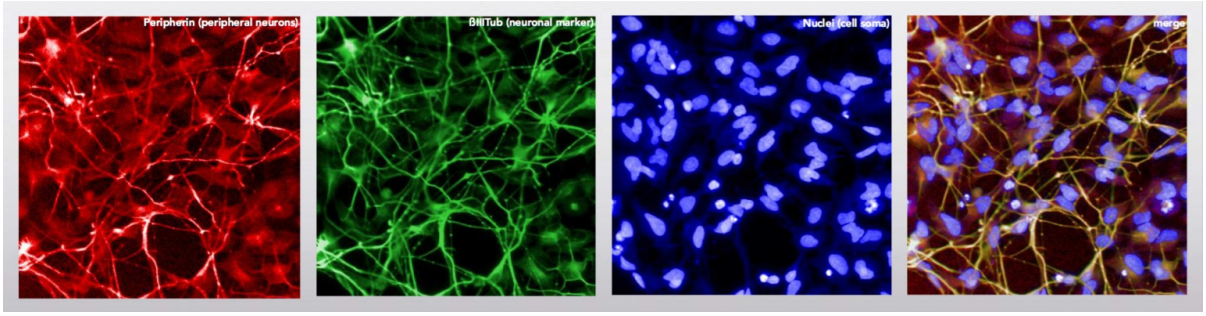
Topic: D.02. Somatosensation: Pain

Title: human iPSC-derived neurons to address peripheral concerns: applications beyond neurotoxicity assessment

Authors: G. LUERMAN[1], D. HESS[2], *B. MURPHY[1], A. EHLICH[2], H. BOHLEN[2]; [1]Axiogenesis, Plymouth Meeting, PA; [2]Axiogenesis AG, Cologne, Germany

Abstract: New technologies are needed to address gaps in drug development, not only for neurodegenerative disorders and neurotoxicological liability screens, but also for numerous other

clinically related applications (e.g. BoNT screening, pain, etc). Historically, drug development and toxicology screens have employed classical animal derived *in vitro* and *ex/in vivo* models, but have lacked relevant human cell models until clinical trials. Thus, preclinical biomarkers may have poor translation to the human clinical condition, low sensitivity, or lack of specificity for neuron-specific effects (e.g. astrogliosis). Additionally, human iPS neurons have largely targeted CNS disorders, while not addressing peripheral related endpoints.
To address these concerns, Axiogenesis developed highly pure human iPSC derived (>80% peripherin positive) neurons. Here we show, using microelectrode array analysis, that Axiogenesis neurons are highly spontaneously active cells characterized by prominent burst activity with high signal/noise. Additionally, these cells demonstrated neurite-specific sensitivity to many common chemotherapeutics associated with clinical peripheral neuropathy. Finally, we speculate on the ability of iPS-derived neurons to address pain related endpoints during preclinical development.



Disclosures: **G. Luerman:** A. Employment/Salary (full or part-time): Axiogenesis AG. **D. Hess:** A. Employment/Salary (full or part-time): Axiogenesis AG. **B. Murphy:** A. Employment/Salary (full or part-time): Axiogenesis AG. **A. Ehlich:** A. Employment/Salary (full or part-time): Axiogenesis AG. **H. Bohlen:** A. Employment/Salary (full or part-time): Axiogenesis AG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axiogenesis AG.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.01/JJ7

Topic: D.02. Somatosensation: Pain

Support: NIH R01DA37621 to BKT

Title: Latent pain sensitization is masked by spinal mu and kappa, but not delta, opioid receptor analgesia in male and female mice.

Authors: *L. CUSTODIO-PATSEY, R. R. DONAHUE, W. FU, B. K. TAYLOR;
Univ. of Kentucky, Lexington, KY

Abstract: Tissue or nerve injury elicits a sustained silent form of neuronal plasticity that increases the susceptibility to chronic pain, entitled latent sensitization (LS). Our laboratory previously demonstrated that injury tonically activates spinal μ-opioid receptors, which in turn opposes nociceptive transmission, preventing the transition from acute to chronic pain (Corder et al, Science, 2013). However, whether other opioid receptors contribute to the maintenance of the remission phase of LS remains uncertain. Moreover, preclinical and clinical studies indicate a sexual dimorphism in the responsiveness to the analgesic effects of ƙ-selective agonists. Therefore, we investigated whether there are sex differences in endogenous ƙ- (and also δ- and μ-) opioid receptor-mediated inhibition of LS. To help to answer these questions, we intrathecally administered opioid subtype-selective antagonists after the behavioral hypersensitivity of postoperative pain had resolved, e.g., 21-28 days after surgical incision of the plantar skin plus damage to the plantaris muscle. We found that ƙ-selective antagonists nor-BNI (0.1μg -10μg i.t.) or LY2456302 (0.1μg -10μg i.t.), or the μ-selective antagonist CTOP (0.001μg -0.1μg i.t.), but not the δ-selective antagonists naltrindole (1μg/5μl i.t.) or TIPP [psi] (1μg -10μg i.t.), reinstated pain-like behavior in a dose-dependent manner. Mu and kappa antagonist-induced reinstatement was observed in both male and female mice. There were no significant differences in von Frey thresholds after LY2456302 (10μg i.t.) at 1h (females 0.41 ± 0.11 vs males 0.69 ± 0.21, mean ± SEM), 4h (females 0.41 ± 0.15 vs males 0.69 ± 0.24), and 96 h (females 1.86 ± 0.34 vs males 1.66 ± 0.38) post injection (n=6-7 animals per group); nor on von Frey thresholds after CTOP (0.1μg i.t.) at 30min (females 0.56 ± 0.18 vs males 0.51 ± 0.22), 120min (females 1.23 ± 0.35 vs males 1.04 ± 0.26), and 180min (females 2.00 ± 0.32 vs males 1.80 ± 0.36) post injection (n=8 animals per group). We conclude that mechanical hyperalgesia after plantar incision in the mouse is maintained in state of remission through ƙ- and μ-, but not δ-opioid receptor subtypes, and is sex-independent. We are currently investigating whether lower doses of ƙ- and μ-opioid antagonists will reinstate mechanical or heat hyperalgesia in a sex-dependent manner. We also aim to investigate whether injury up-regulates the expression of opioid receptors on excitatory interneurons within the superficial laminae of dorsal horn.

Disclosures: L. Custodio-Patsey: None. **R.R. Donahue:** None. **W. Fu:** None. **B.K. Taylor:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.02/JJ8

Topic: D.02. Somatosensation: Pain

Title: Agrins may inhibit diabetic neuropathic pain

Authors: *J.-G. CUI[1], D. ERASSO[2], S. ABDI[3];
[1]Dept of PAIN MEDICINE, DIVISION of Anesthesiol., MD ANDERSON CANCER CENTER, Houston, TX; [2]Univ. of Miami, Miami, FL; [3]MD Anderson Cancer Ctr., Houston, TX

Abstract: Neuropathic pain occurs in approximately 50% of diabetic patients, which constitutes 3.2% of the adult population. The mechanisms for neuropathic pain development are complex, involving pathological alterations of gene, neurotransmitters, irons, molecular signaling pathways, and cellular structures. Current medications (e.g., non-steroidal anti-inflammatory drugs, anti-convulsants, anti-depressants, and opioids) for NP often yield unsatisfactory results and are frequently complicated by multiple side effects, including dependency, addiction, and/or abuse. Therefore, there is a pressing need to seek new therapy for NP. Agrin proteins play an important role in pain modulation, which can selectively activate GABA neurons' NMDA receptor NR1 subunits to inhibit sensory neuron excitation with specific associated helper molecules mitofusin 2 and neurofilament 135. In this project, we used a STZ induced diabetic neuropathic pain rat model to assess roles of Agr25, Agr50, and Agr75 in diabetic neuropathic pain. STZ was dissolved in 0.05 M citrate buffer (pH 4.5) at 20 mg/ml. A single STZ intraperitoneal injection of 55 mg/Kg was performed. On the following day, glucose in blood was checked with a glucose meter ACCU-CHEK (Roche, Mannheim, Germany). After STZ IP injection, the rats developed NP within 7-21 days. However, glucose concentrations in blood were not consistent with neuropathic pain development. Agr genes were installed into AAV2 vector and expressed in HEK293 cells. The purified Agr25, Agr50, and Agr75 were injected intrathecally into diabetic neuropathic pain rats. Some of the Agr proteins suppressed neuropathic pain. The experiments are going on.

Disclosures: J. Cui: None. **D. Erasso:** None. **S. Abdi:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.03/JJ9

Topic: D.02. Somatosensation: Pain

Support: Fellowship of CONACYT 277866

Title: Lack of effect of diazepam on the presynaptic depolarization of primary afferents in the undernourished rat.

Authors: *M. G. CARREON[1], C. VELÁZQUEZ-DELGADO[1], B. SEGURA-ALEGRÍA[2], S. QUIROZ-GONZÁLEZ[3], I. JIMENEZ-ESTRADA[1];
[1]Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV-IPN, Mexico city, Mexico; [2]Deparment of Biol., Natl. Autonomous Univ. of Mexico, Mexico city, Mexico; [3]Dept. de Acupunctura y Rehabilitacion, Univ. Estatal del Valle de Ecatepec, Mexico city, Mexico

Abstract: It has been demonstrated that Diazepam (DZP) administration increases the amplitude and duration of the positive component (P wave) in the cord dorsum potential (CDP) and the dorsal root potential (DRP) evoked in the rat (Schmidt et al, 1967). Both potentials are representatives of the primary afferent depolarization (PAD). Quiroz and collaborators (2012) have shown that the electrical stimulation of the cutaneous sural nerve (nSU) generates P waves in CDPs and DRPs of small amplitude in chronic undernourished rats. In this study we analyze the changes in amplitude and duration of the nSU-evoked CDPs and DRPs in control and chronically undernourished Wistar rats (60 days old) by DZP administration (2 mg/kg-bw). In both groups of animals, nSU was stimulated by single current pulses (1Hz; 3 times threshold, 0.2 ms duration). The CDP was recorded on the L6 spinal segment and the DRP on a L6 rootlet. The nSU evoked CDP usually contain 4 components: An afferent volley (AV), two negative components (N1 and N2), and one positive component (P wave).Intraperitoneal administration of DZP to control rats increases the amplitude, area and duration of the N1 component (105.58 ± 4.58% , 171.87 ± 18.35 % and 164.34 ± 13.11 %, respectively) and P wave in the CDP (168.11 ± 17.63 %, 276.80 ± 76.54 % and 296.24 ± 44.54 %, respectively), even in the DRP (125.78 ± 6.94 %, 216.16 ± 97.45 % and 316.79 ± 145.21 %, respectively, p= <0.05. n = 6) Meanwhile, in the undernourished animals DZP administration practically had no effect on the CDP and DRP. The results obtained may suggest that chronic undernutrition alters the expression of the α1, α2 , α3 and γ2 subunits (sensitive to benzodiazepines) in GABAA receptors which are involved on the presynaptic depolarization of primary afferents and presynaptic inhibition in the rat .

Disclosures: M.G. Carreon: None. **C. Velázquez-Delgado:** None. **B. Segura-Alegría:** None. **S. Quiroz-González:** None. **I. Jimenez-Estrada:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.04/JJ10

Topic: D.02. Somatosensation: Pain

Support: NIH R21DA038248

NIH K01DA031961

NIH R01DA37621

Title: Calcium-permeable AMPA receptor signaling in dorsal horn contributes to latent pain sensitization after inflammation

Authors: R. R. DONAHUE[1], G. P. SINHA[1], J. A. MORON[3], B. K. TAYLOR[1], *S. DOOLEN[2]; [1]Physiol., [2]Dept. of Physiol., Univ. of Kentucky, Lexington, KY; [3]Anesthesiol., Washington Univ., Saint Louis, MO

Abstract: Central sensitization (CS) in dorsal horn neurons is thought to contribute to chronic pain states. Less appreciated, however, are data which suggest that CS outlasts overt signs of hyperalgesia, in a silent form termed latent sensitization (LCS). LCS can be revealed with numerous interventions including stress or drugs that reinstate hyperalgesia. For example, we reported in Science (Corder et al, 2013)that the opioid receptor inverse agonist naltrexone (NTX) reinstated pain-like behaviors and signs of spinal neuron activation such as glutamate-evoked Ca^{2+} responses when administered long after the resolution of the initial inflammatory hyperalgesia. Ca^{2+} signaling is crucial in the central sensitization that drives chronic pain. Glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) are critically involved in excitatory synaptic transmission, and acute inflammatory insult (1-3 days) triggers spinal accumulation of calcium permeable (CP) AMPARs that coincides with inflammatory hyperalgesia. However, whether CP AMPARs contribute to LCS is unknown. To test the hypothesis that blockade of opioid receptor activity with naltrexone (NTX) will reinstate CP AMPAR-mediated Ca^{2+} signaling during LCS, we performed 4 studies: 1) We measured the effect of an antagonist of CP AMPAR, Naspm, on NTX-induced behavioral reinstatement 21d after injection of complete Freund's adjuvant (CFA, 5-10 ul, i.pl). von Frey (vF) measures of tactile sensitivity were assessed 3, 7 or 21 d later. Consistent with our previous studies, 1 ug NTX (*i.t.*) reinstated mechanical hyperalgesia. We found that Naspm (1 nmol, *i.t.*) dose-dependently decreased NTX-induced mechanical hypersensitivity; 2) We used live-cell Ca^{2+} imaging in adult mouse transverse lumbar slices (450 um), bulk loaded with 10 uM fura-2 AM to measure Ca^{2+} signals in response to AMPA 21d after CFA injury. We found AMPA-evoked Ca^{2+} signaling was greater in the presence of 30 μm NTX; 3) We used patch-clamp electrophysiology

of lamina II neurons to study AMPA currents at 3 d (coinciding with acute hypersensitivity) and 21 d after CFA (during LCS). Consistent with previous studies, current-voltage curves obtained from spinal slices taken 3 d after injection of CFA but not saline exhibited a marked inward rectification at positive potentials. Similarly, we observed inward rectification 21d after CFA; 4) Preliminary western blot data suggest CP AMPARs remain at the post-synaptic density 3d and 21 d after CFA. These data suggest that synaptic CP AMPARs are increased soon after injury, and remain at the synapse during LCS. Targeting CP AMPARs may provide relief in chronic inflammatory pain states.

Disclosures: R.R. Donahue: None. **G.P. Sinha:** None. **J.A. Moron:** None. **B.K. Taylor:** None. **S. Doolen:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.05/JJ11

Topic: D.02. Somatosensation: Pain

Support: NIH Grant DA015353

NIH Grant Al113580

Title: Effects of botulinum toxin on histamine-dependent and histamine-independent pruritus

Authors: *T. L. YAKSH[1], M. J. MARINO[1], S. PAUL[1], Z. WANG[2], N. MASCARENHAS[2], A. DINARDO[2], R. RAMACHANDRAN[1];
[1]Univ. of California San Diego, La Jolla, CA; [2]Dermatol., UCSD, La Jolla, CA

Abstract: Background: Pruriceptive itch as seen in atopic dermatitis originates following the activation of peripheral sensory nerve terminals associated with allergic reactions either induced by insect bites or when pruitogens come in contact with the skin. The transmission of the pruriceptive signals is mediated by the release of neurotransmitters or peptides from the primary afferents. The ability of botulinum neurotoxins (BoNTs) in attenuating neurotransmitter release has been extensively used in clinic in treating several pain disorders thus, providing a rationale for its application in pruritus. Therefore, the present study investigates the effects of BoNT-A1 and BoNT-B1 serotypes on Compound 48/80 and Chloroquine (CQ) induced itch.
Method: C57Bl/6 mice (male, 25–30 gram) were shaven on the dorsolateral aspect of the neck and upper shoulder. The detection band was placed around the hind paw ipsilateral to the shaven area. Animals are then adapted to the testing chambers for 1 hour. To initiate scratching

behavior, intradermal (ID) injection of the compound 48/80 or CQ was made in the middle of the shaven area of skin using a 30gauge needle and the scratching behavior was recorded using the paw motion detector (PWD) for a period of 40 min. To determine the effects of BoNTs on compound 48/80 and CQ induced scratching, unilateral injections of 1.5 U of BoNT-A or BoNT-B or saline were given 2, 7, 14 and 21 days prior to ipsilateral compound 48/80 and CQ treatment

Results: A significant increase in the bouts of scratching was observed following intradermal injection of histamine dependent compound 48/80 and histamine-independent CQ. Pre-treatment with BoNT-A1 and BoNT-B1 significantly reduced compound 48/80 induced scratching behavior on day 2, 7 and 14 but not on day 21 as compared to the saline treated group suggesting a reversal of BoNT effect by day 21. A similar long lasting effect of BoNT-A1 and B1 were observed on CQ induced scratching as well. BoNT-A1 and BoNT-B1 groups were not statistically different from each other either in compound 48/80 or CQ treated groups.

Discussion: The present study for the first time demonstrated the anti-pruritic effects of two well characterized and clinically employed BoNT serotypes, Botulinum Toxin A1 (A: BoTox©) and Botulinum Toxin B (B: MyoBloc©) over time in pruritus model using two different mechanistically driven pruritogens.

Disclosures: T.L. Yaksh: None. **M.J. Marino:** None. **S. Paul:** None. **Z. Wang:** None. **N. Mascarenhas:** None. **A. DiNardo:** None. **R. Ramachandran:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.06/JJ12

Topic: D.02. Somatosensation: Pain

Support: MR/K022539/1

Title: New botulinum conjugates targeting NK1 and opiate receptor expressing neurons for the control of chronic pain

Authors: S. M. GERANTON[1], M. MAIARU[1], A. S. MANGIONE[1], C. TASSORELLI[2], E. FERRARI[3], C. LEESE[4], B. DAVLETOV[4], *S. P. HUNT[1];
[1]Univ. Col. London, London, United Kingdom; [2]"C. Mondino" Natl. Neurolog. Inst., Pavia, Italy; [3]Univ. of Lincoln, Lincoln, United Kingdom; [4]Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Botulinum neurotoxin/A (BoNT/A) reversibly blocks neuromuscular synaptic transmission by cleavage of the specific target SNAP25 essential for neurotransmitter release. However, there is also growing evidence that BoNT/A will ameliorate chronic pain states although the mechanism remains unclear. Recently, a botulinum A-based molecule (BiTOX) has been developed that maintains neuronal silencing capacity and reduces peripheral neuropathic pain states but without muscle paralysis (Mangione et al.,2016). Here we have extended this approach by conjugating the silencing domain of BoNT/A (BOT) to either substance P or dermorphin to reversibly inhibit key NK1 or mu-opiate receptor-expressing neurons in the pain pathways and ameliorate chronic pain states.
SP-BOT or Derm-BOT conjugates were injected intrathecally in mice either 2 weeks prior to surgery or 5 days after surgery for the spared nerve injury model of neuropathic pain (SNI). Von Frey's filaments were used to monitor mechanical sensitivity. The targeting of conjugates was monitored using immunochemistry. Lumbar spinal cord sections were stained for cleaved SNAP25 using a specific antibody and tyramide amplification followed by mu-opiate receptor antibody or NK1-rceptor antibody.
SP-BOT (100ng/2µl) treatment before surgery attenuated the increase in mechanical sensitivity seen after SNI (55%). Similarly, when the construct was injected after SNI surgery SP-BOT injected mice showed reduced neuropathic sensitivity compared with the vehicle-injected SNI mice (40%). Derm-BOT conjugates (100ng/3µl) also reduced mechanical hypersensitivity when injected intrathecally in mice after SNI surgery. Immunohistochemistry demonstrated subsets of cleaved SNAP 25-positive neurons in the dorsal horn of conjugate injected mice. Double staining with either NK1 or mu-receptor antibodies indicated that up take of conjugates was largely specific: SP-BOT was found in NK1 positive neurons and Derm-SAP in mu-receptor positive neurons.
In conclusion, the data strongly suggests that both BOT constructs have translational potential for the treatment of chronic pain by selectively and reversibly silencing central neurons involved in pain processing.

Disclosures: S.M. Geranton: None. **M. Maiaru:** None. **A.S. Mangione:** None. **C. Tassorelli:** None. **E. Ferrari:** None. **C. Leese:** None. **B. Davletov:** None. **S.P. Hunt:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.07/JJ13

Topic: D.02. Somatosensation: Pain

Support: Department of Anesthesiology, Stony Brook Medicine

Title: Inhibition of transient receptor potential vanilloid type 1 (TRPV1) receptor by α2-adrenergic receptors in dorsal root ganglia neurons

Authors: *M. PUOPOLO, S. CHAKRABORTY, Y. LU, M. REBECCHI;
Anesthesiol., Stony Brook Med., Stony Brook, NY

Abstract: The TRPV1 channel is a molecular integrator in the pain pathway preferentially expressed in Aδ- and C-fiber nociceptors. Expression on the central terminals of nociceptors make TRPV1 channels well positioned to interact with neuromodulators released in the dorsal horn of the spinal cord by supraspinal centers. The aim of this project was to investigate whether norepinephrine (NE) could modulate the activity of TRPV1 channels expressed in nociceptors. Experiments were carried out in isolated dorsal root ganglia (DRG) neurons and in horizontal spinal cord slices from P18-28 rats using the patch clamp technique. In DRG neurons, the TRPV1 current was isolated as the capsaicin-activated current during a ramp of voltage from -100 to +100 mV. The external solution was (mM): 151 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES. The internal solution was (mM): 125 CsCl, 10 NaCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 4 MgATP, 0.3 NaGTP, 14 phosphocreatine, pH=7.2. In spinal cord slices, miniature excitatory postsynaptic currents (mEPSCs) were recorded in voltage clamp (V_h= -70 mV) from lamina I neurons. The external solution was (mM): 125 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4.H_2O$, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 20 Glucose, 0.01 bicuculline, 0.005 strychnine, supplemented with 300 nM tetrodotoxin and 200 nM ω-Con-MVIIC. The internal solution was (mM): 125 $CsMeSO_3$, 10 NaCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 4 MgATP, 0.6 GDP-β-S, 14 phosphocreatine, 5 QX-314, pH=7.2. In small DRG neurons, 1 μM NE inhibited the capsaicin-activated inward current by 45±9% (n=11). 1 μM clonidine had similar effects (inhibition by 54±10%, n=6), suggesting modulation of TRPV1 channels by α2-adrenergic receptors. Removal of external Ca^{2+} reduced the inhibitory effect of 1 μM NE on the capsaicin-activated inward current to only 8±3% (n=10). The inhibitory effect of 1 μM NE on the capsaicin-activated inward current remained unchanged when the activity of PKA or PKC was blocked with H89 or BIM, respectively. In contrast, when the activity of CaMKII was blocked with KN-93, 1 μM NE inhibited the capsaicin-activated inward current by only 12±7% (n=12). In spinal cord slices, 500 nM capsaicin increased the frequency of mEPSCs from 11±3 to 30±12 Hz, and 1 μM clonidine on top of capsaicin reduced the frequency of mEPSCs to 12±5 Hz (n=6), consistent with inhibition of TRPV1 channels mediated by activation of presynaptic α2-adrenergic receptors. The data presented here suggest that: 1) activation of presynaptic α2-adrenergic receptors down regulates the activity of TRPV1 channels; 2) the effect of NE is dependent on Ca^{2+} influx; 3) the intracellular pathway downstream to α2-adrenergic receptors activation is coupled, in large part, to CaMKII activity.

Disclosures: **M. Puopolo:** None. **S. Chakraborty:** None. **Y. Lu:** None. **M. Rebecchi:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.08/JJ14

Topic: D.02. Somatosensation: Pain

Support: NIH NS45954

DA37621

Title: Tonic inhibition of spinal PKA- and Epac-mediated latent pain sensitization by neuropeptide Y

Authors: *W. FU[1], N. YE[2], J. ZHOU[2], B. K. TAYLOR[1];
[1]Physiol., Univ. of Kentucky, Lexington, KY; [2]Dept. of pharmacology and Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Our new models of chronic pain in rodents (Solway et al, PNAS, 2011; Corder et al, SCIENCE, 2013) and humans (Pereira et al, PLoS ONE, 2015) indicate that inflammation or nerve injury produces a silent, or latent sensitization (LS) of nociceptive neurons in the dorsal horn lasting several months, after overt signs of hyperalgesia have resolved. After tissue injury (intraplantar CFA model) or peripheral nerve injury (a mild version of spared nerve injury, CpxSx), LS is under the tonic inhibitory control of endogenous spinal neuropeptide Y (NPY), as indicated by our previous studies showing that intrathecal administration of a selective antagonist of the NPY Y1 receptor, BIBO3304, reinstated pain-like behavior in a dose-dependent manner when injected during the remission phase of LS. To determine the cellular signaling mechanisms underlying the LS that is modulated by Y1 activation, we used pharmacological inhibitors, activators, and transgenic mouse models in the setting of BIBO3304-induced pain reinstatement. We found that BIBO3304 reinstatement was absent in mice lacking adenylyl cyclase 1 (AC1), and could be prevented with intrathecal administration of an AC1 inhibitor (NB001), an activity-dependent NMDAR blocker (MK801), and a TRPA1 antagonist (HC030031). Therefore, we next tested the hypothesis that endogenous NPY/Y1 signaling silences the spinal sensitization driven by cAMP effector proteins. We found that the protein kinase A (PKA) activator (6Bnz) reinstated pain-like behavior when administered during remission; conversely, a PKA inhibitor (H89) attenuated BIBO3304-induced behavioral reinstatement. HC030031 reversed 6Bnz reinstatement. Next, we intrathecally administered one of several inhibitors of exchange protein activated by cAMP (Epac), including ESI-09, HJC0350, and HJC0197 during remission. Each Epac inhibitor attenuated BIBO3304-induced behavioral reinstatement. We conclude that injury sensitizes NMDAR-AC1-Epac and NMDAR-AC1-PKA-TRPA1 pain signaling pathways, both of which are latent due to inhibitory NPY-Y1 signaling. Our data suggest that Y1R signaling is part of an endogenous braking mechanism that silences the behavioral manifestations of chronic

pain, such that mammals naturally recover from the LS associated with inflammation or nerve injury.

Disclosures: **W. Fu:** None. **N. Ye:** None. **J. Zhou:** None. **B.K. Taylor:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 427.09/JJ15

Topic: D.02. Somatosensation: Pain

Support: Grant-in-Aid for Scientific Research (C) (Grant number: 15K10555)

Title: Lidocaine metabolite, monoethylglycinexylidide, affects synaptic transmission in rat spinal dorsal horn

Authors: ***K. FURUTANI**, Y. KAMIYA, T. KOHNO, H. BABA;
Niigata university, Niigata-city, Japan

Abstract: Background: Analgesic mechanisms of systemic administration of lidocaine (Lid) have not fully elucidated. In recent years, some investigators reported that Lid metabolites could exert analgesic action. Therefore, we hypothesized that the one of the Lid metabolites, monoethylglycinexylidide (MEGX), had the analgesic action on spinal dorsal horn. The purpose of this study was to analyze whether MEGX affected synaptic transmission in rat dorsal horn neurons using whole-cell patch clamp recording.
Methods: Adult male Wistar rats were anesthetized by urethane. Thereafter, dorsal laminectomy was performed and lumbosacral segment of spinal cord with ventral and dorsal root was removed. A transverse slice was cut on a microslicer and placed on a nylon mesh in the recording chamber. The slice was perfused continuously with Krebs solution. The effects of MEGX (10 μM) and Lid (10 μM) on the spontaneous excitatory postsynaptic currents (EPSC) and inhibitory postsynaptic currents (IPSC) were examined in lamina II neurons using the whole-cell patch-clamp technique. Data were expressed mean +/- SD. Statistical analysis was performed using Student's *t*-test.
Results: MEGX (10 μM) decreased the frequencies of spontaneous EPSC (44 +/- 22% of control, $p < 0.001$, $n = 7$) with the inward currents (the average amplitude: 13 +/- 6 pA) without affecting the amplitudes. In contrast, MEGX (10 μM) decreased the frequencies of spontaneous IPSC (55 +/- 24% of control, $p < 0.001$, $n = 12$), but biphasic changes (increase followed by decrease) in frequencies were observed in 6 of 12 cells. Outward currents (the average amplitude: 56 +/- 48 pA) were observed in 6 of 12 cells. MEGX did not affect the amplitudes of spontaneous IPSC.

However, Lid (10 μM) did not affect the amplitudes and frequencies of both EPSC and IPSC. Conclusions: MEGX has the possibility to inhibit the presynaptic release of excitatory neurotransmitters. However, MEGX also temporarily increased the frequencies of IPSC and exhibited outward currents in 50% of recording cells, suggesting that MEGX might partially facilitate the release of inhibitory neurotransmitters. In contrast, Lid at clinically-relevant concentration had no effects on synaptic transmission. The analgesic mechanisms of systemic administration of Lid may be exerted by its metabolites.

Disclosures: **K. Furutani:** None. **Y. Kamiya:** None. **T. Kohno:** None. **H. Baba:** None.

Poster

427. Spinal Cord Processing: Pharmacology

Location: Halls B-H

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Title: Molecular and electrophysiological characterization of neuropeptide Y Y1 receptor-expressing neurons in the substantia gelatinosa of the spinal cord

Authors: ***G. P. SINHA**[1], W. FU[2], K. C. HALMOS[2], B. N. SMITH[2], S. DOOLEN[2], B. K. TAYLOR[2];
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Abstract: Neuropeptide Y (NPY) receptors are expressed in *substantia gelatinosa* (lamina II) neurons of the spinal cord. NPY reduces behavioral signs of acute and chronic pain, in part through activation of the NPY Y1 receptor (Y1R). However, the cellular mechanism of Y1R-mediated analgesia remains unclear. One outstanding question is whether they are expressed in inhibitory and/or excitatory neurons, and where they fit within the dorsal horn microcircuity of pain transmission and pain control, especially in the setting of chronic pain arising from tissue or nerve injury. Behavioral pharmacology and targeted neurotoxin studies from our laboratory support the hypothesis that Y1R-expressing neurons are excitatory. Furthermore, we report that Y1Rs co-exist with multiple markers of excitatory neurons such as Tlx3, calbindin, calretinin,

and somatostatin, but not a widely accepted marker of spinal inhibitory interneurons, PAX2. Using patch-clamp electrophysiology in current clamp mode we recorded from lamina II neurons in para-sagittal slices from the spinal cord lumbar L4/L5 segment of adult mice. In randomly recorded unlabeled neurons, we observed firing patterns in the following ratios: tonic (35 %), initial burst (28 %), delayed and gap (15 %) and single (22 %). In Y1R- positive cells visualized from slices prepared from Y1R-eGFP mice, the majority of cells exhibited initial burst firing (90%) upon current injection, with a small percentage (10%) exhibiting tonic firing. It is widely assumed that tonic and perhaps initial burst firing patterns represent inhibitory, GABAergic neurons, and preliminary single-cell PCR experiments in Y1-expressing neurons using GAD67 and vGlut2 probes seem to confirm this. Also, dorsal root stimulation (DRS) evoked monosynaptic (putative) EPSCs at A-δ recruiting strengths in Y1R-GFP neurons (n = 2), suggesting that they receive primary afferent input from A-δ sensory neurons. To determine the effects of NPY on Y1R-GFP neurons, we administered NPY via puff application and observed outward currents (n = 4). In current clamp mode, DRS evoked action potentials were abolished by application of NPY, and this was accompanied by a hyperpolarizing shift of the resting membrane potential by ~10 mV. Both recovered after washout. Taken together, our data suggest that endogenous spinal NPY produces analgesia by altering a complex dorsal horn microcircuit in the dorsal horn that includes interneurons that express Y1R.

Disclosures: **G.P. Sinha:** None. **W. Fu:** None. **K.C. Halmos:** None. **B.N. Smith:** None. **S. Doolen:** None. **B.K. Taylor:** None.

Poster

427. Spinal Cord Processing: Pharmacology

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Topic: D.02. Somatosensation: Pain

Title: Underlying mechanisms of acetaminophen in the spinal dorsal horn neurons

Authors: N. OHASHI[1], M. SASAKI[1], M. OHASHI[1], Y. KAMIYA[2], *H. BABA[3], T. KOHNO[1]; [1]Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan; [2]Uonuma institute of community medicine, Minami-Uonuma, Japan; [3]Niigata Univ, Sch. Med., Niigata, Japan

Abstract: Acetaminophen is one of the most popular and has been widely used as an analgesic agent. Several studies suggest that acetaminophen is metabolized to *N*-acylphenolamine (AM404), which directly acts transient receptor potential vanilloid 1 (TRPV1) and cannabinoid (CB1) receptors in the brain, resulting in analgesia. TRPV1 and CB1 receptors are both present in the superficial spinal dorsal horn, especially the substantia gelatinosa (SG), which is important

as pain pathway. However, no previous studies have reported the analgesic mechanisms of acetaminophen in the spinal dorsal horn. In the present study, we therefore investigated whether clinical level of acetaminophen produces analgesia in the rat spinal dorsal horn using behavioral measures and electrophysiological methods. First, we investigated the effects of AM404 in spinal cord in the radiant heat test. Intrathecal injection of AM404 (0.1, 0.3, 1 nmol) significantly prolonged the withdrawal latency in a concentration-dependent manner, suggesting that AM404 produces analgesia in the spinal cord directly. We then examined the effects of AM404 on primary afferent Aδ and C fibers using spinal transverse slices with the dorsal root attached by whole-cell patch-clamp recording in SG neurons. Monosynaptic excitatory postsynaptic currents (EPSCs) were evoked by Aδ or C fiber stimulation. Bath applied AM404 (30 μM) significantly decreased the amplitude of monosynaptic EPSCs evoked by C fiber stimulation ($P < 0.01$), but not by Aδ fiber stimulation ($P > 0.05$). These effects disappeared in the presence of capsazepine (TRPV1 receptor antagonist), but not AM251 (CB1 receptor antagonist). Taken together, these findings strongly suggest that AM404 metabolized from acetaminophen produces analgesia through TRPV1 receptors expressed on C fibers in the spinal dorsal horn.

Disclosures: **N. Ohashi:** None. **M. Sasaki:** None. **M. Ohashi:** None. **Y. Kamiya:** None. **H. Baba:** None. **T. Kohno:** None.

Poster

427. Spinal Cord Processing: Pharmacology

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Program#/Poster#: 427.12/KK1

Topic: D.02. Somatosensation: Pain

Support: NHMRC

Title: Effect of voltage gated sodium channel toxins as therapeutic agents for chronic pain

Authors: N. R. MUNASINGHE[1], J. DEUIS[2], V. HERZIG[2], Z. DEKAN[2], *W. L. IMLACH[3], R. LEWIS[2], G. KING[2], P. ALEWOOD[2], J. KLINT[2], I. VETTER[2], M. J. CHRISTIE[1];
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Abstract: In humans, the loss function mutation in the SCN9A gene that codes for voltage gated sodium channel 1.7 ($Na_V1.7$) caused insensitivity to pain, while gain of function mutations resulted in paroxysmal extreme pain disorder and primary erythermalgia. $Na_V1.7$ therefore plays an important role in pain. $Na_V1.7$ channels are abundant in small (<25μm) primary sensory neurons of the dorsal root ganglion (DRG). This project aimed to identify the efficacy of animal

toxins that are selective for $Na_V1.7$, which may serve as potential therapeutic agents in the treatment of pain. In order to identify the role of $Na_V1.7$ channels, whole cell patch clamp electrophysiology was conducted on acutely isolated neurons from male Sprague Dawley rats. Types of DRG neurons were discriminated based on isolectin-B4 binding (peptidergic neurons do not stain) and cell size. The synthetic spider toxin Hs1a inhibited 70% percent of the small peptidergic DRG Na_V neuronal current while only inhibiting about 30% of the small non-peptidergic Na_V neuronal current. Similarly, the synthetic venom peptide 1 was found to be most effective in small peptidergic neurons. When the tetrodotoxin sensitive Na_V current component was isolated, there was about 80-90% inhibition by peptide 1 (1μM) in both peptidergic and non peptidergic, small DRG neurons. Since $Na_V1.7$ is potently blocked by tetrodotoxin, these results show promise that the inhibited Na_V current is primarily due to $Na_V1.7$ blockade. Peptide 1 shows little inhibition in large DRG neurons which are known to express very low levels of $Na_V1.7$. In spinal cord slices, peptide 1 inhibited electrically-evoked afferent transmission (at C-fibre strength) onto dorsal horn neurons. Overall, these results support both Hs1a and peptide 1 as having therapeutic potential to treat pain by selectively inhibiting $Na_V1.7$ in nociceptive neurons.

Disclosures: **N.R. Munasinghe:** None. **J. Deuis:** None. **V. Herzig:** None. **Z. Dekan:** None. **W.L. Imlach:** None. **R. Lewis:** None. **G. King:** None. **P. Alewood:** None. **J. Klint:** None. **I. Vetter:** None. **M.J. Christie:** None.

Poster

427. Spinal Cord Processing: Pharmacology

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Title: Peripheral nerve injury increases stimulation-induced neuropeptide Y release as measured by Y1 receptor internalization in the rat dorsal horn

Authors: *B. K. TAYLOR[1], W. FU[1], W.-L. CHEN[2], J. G. MARVIZON[2];
[1]Physiol., Univ. of Kentucky, Lexington, KY; [2]UC Los Angeles, VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: Peripheral nerve injury greatly increases the expression of neuropeptide Y (NPY) in myelinated afferents and their central terminals in laminae II-IV of dorsal horn. We previously reported that tissue injury increases NPY expression, NPY Y1 receptor (Y1) signaling, and spinal anti-hyperalgesia (Taylor et al., Neuroscience, 2014). However, whether this is associated with an increased release of NPY and Y1 activation has been difficult to study in the absence of a sensitive and reliable assay. Therefore, we developed Y1R internalization as a new *in situ* assay of spinal NPY release. This approach is similar to the extensive use of neurokinin 1 receptor and μ-opioid receptor internalization to measure the release of substance P and opioids, respectively. Receptor internalization has several advantages over other methods to measure neuropeptide release such as microdialysis, antibody microprobes and peptide immunoassays: 1) it allows the localization of the areas of release (segment, side, lamina, etc.); 2) it is an *in situ* measure and thus avoids problems with extracting the peptide; 3) it is sensitive; 4) it is non-invasive; 5) it detects peptide release that is physiologically relevant in terms of receptor activation. We found that NPY concentration-dependently induced Y1R internalization in rat spinal cord slices with and EC_{50} of 43 nM (95% CI 18-100 nM). In spinal cord slices, electrical stimulation of the dorsal horn (1000 pulses of 20 V, 0.4 ms, at 5 Hz and 500 Hz) induced Y1R internalization. In contrast, the same 1000 pulses delivered to the dorsal root (at 5 Hz or 100 Hz) failed to induce a significant amount of Y1R internalization. Y1R internalization induced by dorsal horn stimulation increased 14 days after spared nerve injury (SNI). Next, we substituted electrical stimulation of the dorsal horn with stimulation of the sural receptive field of the plantar skin ipsilateral to SNI. At day 14 after SNI, rats were anesthetized with isoflurane and received a non-noxious (gentle stroke with the experimenter's thumb for 2 sec, repeated every 4 sec for 2 min) or noxious stimulus (application of a 2-cm wide binder clip for 2 min). Rats were euthanized 5 minutes later and used to measure Y1R internalization in the lumbar spinal cord. We found that both non-noxious and noxious stimulation markedly increased Y1R internalization in the dorsal horn ipsilateral but not contralateral to SNI. Therefore, SNI increases NPY release induced by electrical stimulation of the dorsal horn in slices or by sensory stimulation of the hindpaw. We conclude that after nerve injury, sensory stimulation evokes NPY release from A-fibers or from neurons receiving synapses from them.

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Poster

427. Spinal Cord Processing: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: MR/K015052/1

Title: Inhibition of bone resorption does not contribute to the analgesic effects of Src antagonism in an animal model of cancer-induced bone pain

Authors: *V. HURST[1], D. LAMBERT[2], I. HOLEN[3], K. J. ESCOTT[4], D. ANDREW[1]; [1]OMFS, [2]Biochem. and Cell Biol., [3]Oncology and Metabolism, The Univ. of Sheffield, Sheffield, United Kingdom; [4]Astrazeneca, London, United Kingdom

Abstract: Cancer-Induced Bone Pain (CIBP) is a common and debilitating symptom of patients with advanced disease, and severely reduces their quality of life. Current medical treatments can cause adverse effects or may not produce a significant improvement in symptoms. One proposed mechanism-based therapy is the inhibition of the non-receptor tryrosine kinase Src, which controls N-Methyl-D-Aspartate receptor (NMDAR) activity. The NMDAR plays a key role in the pathophysiology of pain and inhibiting Src is effective in reducing pain in a rat model of CIBP (de Felice et al. 2016). Cancer patients with bone metastases receive bisphosphonates such as zoledronic acid (ZOL) to prevent skeletal related events, such as pathological fractures, spinal cord compression and hypercalcaemia. ZOL also has weak analgesic efficacy as shown in a meta-analysis (Wong & Wiffen 2002). We have investigated whether co-administration of a clinically-relevant dose of ZOL with the Src-inhibitor Saracatinib increased the magnitude of hyperalgesia inhibition in a rat model of CIBP, and whether combined treatment also improved bone preservation. Thirty-six male Sprague-Dawley rats were injected with $6x10^4$ MRMT-1 (rat mammary cancer; Riken, Japan) cells into the left tibia. Seven days later the animals were given a single injection of ZOL (100ug/kg s.c.) or vehicle, and administered either Saracatinib (20mg/kg) or vehicle daily for one week by gavage. Mechanical allodynia and thermal hyperalgesia of the plantar surface of the hind limb paws were measured pre- and post-surgery. The tumour-bearing bones and serum were harvested at day 14 post-surgery for microtomography, histology, and to measure markers of osteolysis. Animals administered Saracatinib (either alone or with ZOL) showed reduced thermal hyperalgesia as compared with animals treated with vehicle alone. There was no effect of ZOL on pain behaviour, nor any additive effects when administered with Saracatinib. Bone resorption markers were reduced and bone volume increased in animals treated with combined ZOL + Saracatinib, but not Saracatinib alone. These data indicate that administration of a clinically-relevant dose of ZOL in combination with Saracatinib does not provide any therapeutic gain in pain relief or bone-preservation.

Disclosures: V. Hurst: None. **D. Lambert:** None. **I. Holen:** None. **K.J. Escott:** A. Employment/Salary (full or part-time): employee of Astrazeneca. **D. Andrew:** None.

Poster

427. Spinal Cord Processing: Pharmacology

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Support: NIH R01NS62306 and NIH P30DK020579/University of Kentucky-Washington University Diabetes Research Center Collaborative Pilot & Feasibility Award to BKT

Title: Sex differences in pioglitazone analgesia for painful diabetic neuropathy

Authors: *D. E. LAIRD, R. R. DONAHUE, R. B. GRIGGS, B. K. TAYLOR;
Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Diabetes affects 9% of the United States population equating to over 25 million adults and children. Approximately one-half of patients with diabetes experience neuropathy, which is characterized by motor, autonomic, and sensory nerve dysfunction. Approximately two-thirds of patients with neuropathy experience pain, commonly referred to as painful diabetic neuropathy (PDN). Maintaining blood glucose in the normal range is first line of defense against PDN. In late-stage type 2 diabetes, however, PDN is difficult to manage. Blood levels of methylglyoxal (MG), a highly reactive dicarbonyl product of glycolysis that accumulates in plasma during hyperglycemia, is elevated in patients with diabetes, and even further elevated in patients with PDN. We found elevated levels of MG in plasma of the ZDF and db/db rodent model PDN. Also, intraplantar injection of MG produced dose-dependent pain-like behaviors in mice and rats (e.g. licking and lifting of the hind paw), hyperalgesia (mechanical and heat hypersensitivity), conditioned place aversion and pERK in superficial laminae of the dorsal horn. PPARγ agonists such as pioglitazone have analgesic properties distinct from their antidiabetic properties. Since PPARγ agonists are already available and have proven beneficial effects in diabetes in humans, testing their mechanisms of action in animal models seems like a reasonable approach. We are currently investigating the therapeutic effects of pioglitazone on MG-induced thermal hypersensitivity in males and females using a hot plate assay, as well as MG-induced mechanical hypersensitivity using von Frey filaments. We found that pretreatment with 100 mg/kg i.p. pioglitazone, but neither 10 mg/kg nor saline, prevented MG-induced (100 µg / 5µl i.pl.) mechanical hypersensitivity in male C57Bl/6 mice. By contrast, 10 mg/kg pioglitazone substantially reduced the development of MG-induced mechanical hypersensitivity in females. Preliminary dose-response data from the lab indicate that low doses (1, 10 mg/kg) of pioglitazone inhibit MG-induced thermal and mechanical hyperalgesia in female but not male mice. These data suggest that pioglitazone is more effective in reducing MG-induced thermal and mechanical hyperalgesia in female than in male mice. Further studies are needed to determine whether this sex difference extends to models of PDN.

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Poster

427. Spinal Cord Processing: Pharmacology

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Title: Small molecule inhibitors of PSD95-nNOS protein-protein interactions suppress formalin-evoked Fos protein expression and nociceptive behavior in rats.

Authors: *L. M. CAREY, IV[1], P. KULKARNI[2], G. A. THAKUR[2], Y. Y. LAI[1], A. G. HOHMANN[1];
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Abstract: Excessive activation of NMDA receptor (NMDAR) signaling within the spinal cord contributes to the generation of central sensitization, a phenomenon which underlies the induction and maintenance of pathological pain states. However, direct antagonism of NMDARs produces a wide range of undesirable side effects (e.g. impairments in learning, memory and motor function) which limit their clinical use. NMDAR activation initiates central sensitization, at least in part, by initiating a signaling cascade that activates the enzyme neuronal nitric oxide synthase (nNOS) and generates the signaling molecule nitric oxide (NO). NMDAR activation of nNOS requires a scaffolding protein, postsynaptic density protein 95 kDa (PSD95), which tethers nNOS to NMDARs. Thus, disrupting the interaction between PSD95 and nNOS may inhibit pro-nociceptive signaling mechanisms downstream of NMDARs and suppress central sensitization while sparing unwanted side effects associated with direct NMDAR antagonists. We examined the impact of two small molecule PSD95-nNOS protein-protein interaction inhibitors, ZL006 and IC87201, on formalin-evoked nociceptive behavior as well as Fos protein expression, a marker of neuronal activation, within the lumbar spinal dorsal horn, using the same subjects. To assess specificity of small molecule inhibitors, comparisons were made with ZL007, an analog of ZL006 that does not disrupt PSD95-nNOS interactions in vivo. The noncompetitive NMDAR antagonist MK-801 was used as a positive control. IC87201 and ZL006 selectively suppressed

phase 2 of formalin-evoked pain behavior whereas ZL007 did not. Moreover, IC87201 and ZL006 similarly decreased the number of Fos-like immunoreactive cells in lumbar spinal cord regions associated with nociceptive processing. PSD95-nNOS inhibitors suppressed the number of formalin-evoked Fos like immunoreactive cells in the superficial dorsal horn (laminae I, II), the nucleus proprius (laminae III, IV) and the neck region of the dorsal horn (laminae V, VI). The pattern of biochemical and behavioral changes induced by PSD95-nNOS inhibitors was also similar to that produced by MK801. The present findings validate the use of small molecule PSD95-nNOS protein-protein interaction inhibitors as novel analgesics and demonstrate, for the first time, that these inhibitors suppress inflammation-evoked neuronal activation at the level of the spinal dorsal horn.

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Poster

427. Spinal Cord Processing: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: The National Natural Science Foundation of China (No. 81260175 and 81560198)

Title: Contribution of presynaptic HCN channels to excitatory inputs of spinal substantia gelatinosa neurons

Authors: ***T. LIU**[1], S. PENG[2], D. ZHANG[2], X. HU[2], L. LI[2], C. XIE[2];
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Abstract: Aim of Investigation: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are widely distributed in nervous system and are associated with neuropathic pain. HCN channel family comprises four homologous isoforms (HCN1-HCN4). Activation of HCN channel results in an inward mixed cation current. Lamina II in spinal cord (also named substantia gelatinosa, SG) is identified as the primary center to regulate peripheral nociceptive signal, where the excitatory interneurons dominate sensory processing. In order to know in detail about the contribution of HCN channels in regulating nociceptive transmission, the expression of HCN channel isoforms in glutamatergic synaptic terminal and the role of presynaptic HCN channels to excitatory inputs in spinal SG were investigated.**Methods:** We examined the expression of HCN1-HCN4 isoforms in presynaptic terminal using the immunohistochemical

technology. Whole-cell patch-clamp recordings were applied to SG neurons in spinal cord slices prepared from both male juvenile SD rats and GAD67-GFP transgenic C57BL6 mice. Spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs) are recorded in whole-cell mode at a holding potential of -70 mV. **Results:** All isoforms of HCN channels were found in superficial spinal dorsal horn. Among them, HCN4 was partly co-expressed with VGLUT2 (marker of excitatory presynaptic terminal). Unlike HCN4, HCN1-HCN3 were seldom co-expressed with VGLUT2. Superfusion of an HCN channel antagonist, ZD7288, dose-dependently decreased the frequency but not the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in nearly 70% of SG neurons examined. Moreover, the ZD7288-induced reduction was mimicked by another HCN channel blocker cesium chloride and not affected by tetrodotoxin. On the contrary, 50 μM of forskolin (the activation of adenylate cyclase) superfused for 15 min significantly increased the frequency but not the amplitude of both sEPSCs and mEPSCs. Interestingly, ZD7288 hardly had effect on sEPSCs of GABAergic SG neurons which labeled with GFP in GAD67-GFP transgenic C57BL6 mice. **Conclusions:** We conclude that HCN isoforms are differently expressed in axon terminal of SG neurons. HCN4 but not HCN1-HCN3 is relatively enriched in excitatory presynaptic terminal. Our data also demonstrate HCN channels are involved in decreasing the release probability of glutamate from presynaptic terminal which innervate excitatory but not inhibitory SG interneurons. Thus, inhibition of HCN4 channels may be a novel pathway for suppression of nociceptive transmission.

Disclosures: **T. Liu:** A. Employment/Salary (full or part-time): full-time. 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 Natural Science Foundation of China. **S. Peng:** None. **D. Zhang:** None. **X. Hu:** None. **L. Li:** None. **C. Xie:** None.

Poster

427. Spinal Cord Processing: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: NRF Grant 2014R1A2A2A01007695

Title: Spinal sigma-1 receptor mediates dephosphorylation of astrocytic aromatase leading to nociceptive effect in mice formalin model

Authors: M.-J. LEE[1], H.-S. CHOI[1], A. J. BEITZ[2], *J.-H. LEE[1];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Vet. and Biomed. Sciences, Col. of Vet. Med., Univ. of Minnesota, St. Paul, MN

Abstract: Aromatase in central nervous system is a key enzyme that produces estrogen from testosterone, which participates in various biological processes including pain sensation. The short term changes of aromatase activity are known to be regulated with rapid and transient cellular processes, such as phosphorylation of proteins at different activation sites. We have previously demonstrated that Sigma-1 receptor, a unique ligand-regulated molecular chaperone, can affect the phosphorylation states of various receptors and enzymes in pain sensation. In this study, we examined whether spinal Sigma-1 receptor can modulate the activity of aromatase through modifying phosphorylation states of aromatase, leading to nociceptive effect in formalin model. Intrathecal (i.t.) injection of letrozole, an aromatase inhibitor, has dose-dependently reduced the nociceptive responses in the second phase of formalin test and the expression of spinal Fos, as compared with those of control group. After showing that aromatase was co-localized with Sigma-1 receptor in spinal astrocytes, we confirmed through co-immunoprecipitation that the level of phosphorylated serine in spinal aromatase was down-regulated in formalin injected control mice. However, this dephosphorylation of aromatase was significantly restored by i.t. administration of BD-1047. Moreover, sub-effective doses of letrozole and BD-1047 showed anti-nociceptive effect when they were co-administered in formalin injected mice. These results demonstrate that Sigma-1 receptor mediated dephosphorylation of astrocytic aromatase indeed induces nociceptive effect in formalin mice, suggesting Sigma-1 receptor as an important factor for aromatase activity modulation in the spinal cord.

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Poster

427. Spinal Cord Processing: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: R01 DA035931

Title: Ifenprodil and agmatine inhibit C-fiber-mediated EPSCs in spinal cord slices from Nav1.8-ChR2 mice.

Authors: J. J. WAATAJA[1], P. A. SÉGUÉLA[3], G. L. WILCOX[1], *C. A. FAIRBANKS[2]; [1]Dept of Neurosci., [2]Depts Pharmaceut, Pharmacol & Neurosci, Univ. Minnesota, Minneapolis, MN; [3]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada

Abstract: Background: Agmatine (decarboxylated arginine) is found throughout the CNS and has been shown to play a role in synaptic plasticity. In the spinal cord, changes in the synaptic strength between nociceptive afferents and their neuronal targets are thought to play a role in the initiation of neuropathic pain. Indeed, exogenous application of agmatine has been shown to attenuate hyperalgesisa (Fairbanks et al., PNAS 2000), spinal opioid tolerance, and inhibit spinal LTP Waataja, SfN Abstracts (2014). Little is known, however, of agmatine's effect on specific subunits of the NMDA receptor in spinal cord dorsal horn.
Methods: Transverse spinal cord slices with a 4-7 mm L4 dorsal root attached were prepared from naïve mice with transgenically introduced channel rhodopsin 2 in Nav1.8-expressing sensory neurons. The dorsal root was stimulated optogenetically by LED-generated 470 nm light applied via the 40x objective of an Olympus BX-50WI microscope to evoke glutamate-mediated excitatory post synaptic currents (eEPSCs); all conduction velocities of afferent fiberss were <1 m/s, confirming that they were all C-fibers. Uhelski-ML and colleagues (SfN Abstracts, 2016) have demonstrated that almost 90% of light-activated neurons in these mice are nociceptors. Dorsal horn neurons were patch-clamped and held at a holding potential of +50 mV to maximize NMDA receptor-mediated EPSCs. We expected to observe EPSCs with two distinct components, faster decay times (~500 ms) indicating the participation of NR2A subunits and slower decay times (~1000 ms) indicating the participation of NR2B subunits; EPSCs were recorded and averaged (N=10). The effect of the NR2B subunit-selective antagonist Ifenprodil (1 - 100 uM) was used as a positive control for NR2B subunit activity and compared against that of agmatine (300 uM - 3 mM).
Results: Bath application of ifenprodil concentration-dependently reduced amplitude, eEPSC recovery time and the tau of eEPSCs recorded from spinal neurons following stimulation. Similarly, bath application of agmatine concentration-dependently reduced amplitude, eEPSC recovery time and the tau of eEPSCs in spinal cord neurons. In both cases, bath application of each compound was sequential resulting in cumulative concentration-response curves.
Conclusion: This study supports the hypothesis that agmatine antagonizes NMDA receptors containing the NR2B subunit. Furthermore, these experiments corroborate that agmatine acts on neurons in the dorsal horn of the spinal cord that receive nociceptive information.

Disclosures: J.J. Waataja: None. **P.A. Séguéla:** None. **G.L. Wilcox:** None. **C.A. Fairbanks:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Support: 2015R1C1A1A01053484

2015021989

Title: Repetitive motor cortex stimulation for the relief of neuropathic pain after nerve injury in rats

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Abstract: Motor cortex stimulation (MCS) is increasingly reported as an effective surgical option for the treatment of neuropathic pain although its mechanism of action remains poorly understood. We hypothesized that analgesic effects of MCS may be related to plastic changes in the anterior cingulate cortex (ACC) in animals with neuropathic pain and, furthermore, the repetitive MCS could alleviate the neuropathic pain more effectively than single MCS. To test this hypothesis, rodent neuropathic pain model were used and the repetitive MCS stimulation (30 min, 50 μA, 50 Hz; 300 ms pulses) was applied during 10 days. The mechanical threshold on the hind paw of the nerve injured rat was recorded before and after repetitive MCS. As results, MCS suppressed the neuropathic pain in nerve-injured rats and repetitive MCS showed the enhanced analgesic effect. In addition, the analgesic effects of repetitive MCS were blocked by inactivation of PKM ζ(related to LTP maintenance) using zeta inhibitory peptide (ZIP). These findings support our hypothesis and suggest that MCS for neuropathic pain is a safe and efficacious treatment option. This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (2015R1C1A1A01053484 and 2015021989).

Disclosures: **M. Cha:** None. **B. Lee:** None.

Poster

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Title: Contribution of ascending serotonin facilitation to neuronal hyperactivity in the anterior cingulate cortex underlying the maintenance of neuropathic pain

Authors: C. BIAN[1], R. HU[1,2], M. LI[1,2], J. LIU[1,3], J.-L. YANG[1], W. GUO[1], S. ZOU[1], K. REN[1], R. DUBNER[1], *F. WEI[1];
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Abstract: The neurotransmitter serotonin (5-HT) in the brain stem modulates neuronal activity in the CNS through its descending and ascending projections. Recent studies have shown that active 5-HT-dependent descending facilitation is involved in central mechanisms underlying the maintenance of neuropathic pain. Although functional changes of the ascending 5-HT system are implicated in the pathophysiology of some affective disorders, such as depression, it is less known whether the ascending 5-HT system contributes to cortical mechanisms of persistent pain. Given the efficacy of tricyclic antidepressants but less benefit of selective 5-HT reuptake inhibitors in the treatment of neuropathic pain, it is necessary to understand how cortical 5-HT activity participates in the modulation of the affective dimension of persistent pain. In the present study, we investigated functional changes of the 5-HT system in the anterior cingulate cortex (ACC) of mice after CCI of the infraorbital nerve (CCI-ION), an animal model of orofacial neuropathic pain, and its contribution to the sensory and affective-emotional components of neuropathic pain. First, a wide-spread and equal density of distribution of 5-HT-IR fibers were observed in all laminae of the ACC. A combination of retrograde tracing and immunostaining demonstrated that ACC 5-HT input came from both the dorsal raphe (DR) and median raphe nuclei (MR). We also observed pERK1/2 expression in 5-HT-IR neurons in both the DR and the MR without variance of 5-HT-IR intensity in the ACC at 14 d after CCI when compared with

naïve animals. Next, using whole-cell patch-clamp recording from ACC slices from naïve and 14d CCI-treated mice, we found that nerve injury caused a significant increase of the frequency and the amplitude of spontaneous and miniature excitatory postsynaptic currents in layer V pyramidal neurons, suggesting CCI-induced neuronal hyperactivity in the ACC. Either bath application of the selective 5-HT3A receptor antagonist, Y25130, or the selective 5-HT1A receptor agonist 8-OH-DPAT alleviated the CCI-induced increase of the frequency but not amplitude of sEPSCs in layer V ACC neurons at 14 d after CCI. Finally, microinjection of 2% lidocaine or Y25130 attenuated mechanical allodynia and negative reinforcement in the conditioned place preference test at 14 d after CCI. Together, these findings suggest that the active ascending 5-HT system enhances excitatory neuronal activity in the deep layer of the ACC and contributes to the maintenance of neuropathic pain conditions by presynaptic 5-HT3AR mechanisms. Reduction of ascending 5-HT facilitation in the ACC may be a potential therapy for neuropathic pain.

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Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

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NIH-GM102691

Title: Chronic pain disrupts the affective response to acute pain through altered neural activities in the anterior cingulate cortex (ACC)

Authors: ***J. WANG**[1], Q. ZHANG[2], A. TONG[2], T. MANDERS[2], A. GARG[2], R. YANG[2], Z. CHEN[2];
[1]Anesthesiol., [2]New York Univ. Sch. of Med., New York, NY

Abstract: Chronic pain is known to impair the affective response to acute pain even at anatomically unrelated locations. The neural substrate for such behavior, however, remains elusive. We hypothesize that the anterior cingulate cortex (ACC), a key brain region for processing the affective component of pain, can play a role. We use a laser to provide acute thermal nociceptive stimuli of varying intensity to the rodent paw. Rodents show an aversion to

the chamber paired with high- vs low-intensity stimulus on the conditioned place aversion (CPA) test, demonstrating an ability to distinguish pain intensity. We also measure local field potentials (LFPs) and spikes in the ACC before, during and after acute pain stimuli in freely moving rats. We find that with increasing pain intensity, there is an increase in the power of LFPs at low frequency ranges. There is also a graded rise in the number of pyramidal neurons that demonstrate increased spike rates after a painful stimulus. Optogenetic activation of the ACC, meanwhile, has the same effect as high-intensity pain stimulus in the CPA test. These results indicate that neurons in the ACC can provide a representation of acute pain intensity. We then test acute pain responses in the chronic pain condition by injecting Complete Freund's Adjuvant (CFA) to the opposite paw. Withdrawal latency in the uninjured paw is not significantly altered, indicating intact peripheral and spinal nociceptive pathway. However, rats with chronic pain are unable to distinguish between low and high stimulus intensity on the CPA, suggesting an impairment in the affective response to acute pain. Correspondingly, in the chronic pain state, ACC neurons display a compressed pain intensity tuning curve. This is demonstrated by increased LFP power and spike rates with low-intensity stimulus but not with non-painful or high-intensity pain stimuli in CFA-treated rats. Finally, our optogenetic studies show that activation of the ACC has the same effect as chronic pain on the CPA test. Together, these results indicate that disrupted ACC circuitry is a major cause for altered response to acute pain in the chronic pain state.

Disclosures: **J. Wang:** None. **Q. Zhang:** None. **A. Tong:** None. **T. Manders:** None. **A. Garg:** None. **R. Yang:** None. **Z. Chen:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Title: Reticular thalamic neuronal activity changes by formalin induced nociception of awake behaving mice

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Abstract: The reticular thalamus (RT), composed mainly of GABAergic neurons, is a structure that wraps around the thalamic structure to provide major inhibitory inputs to thalamic nuclei. Thalamic neurons have characteristic firing mode call the tonic and burst firing mode, and the switch between the two firing mode have been implicated to have roles in modulating sensory information such as vision, touch, and nociception. Of the two firing modes, low threshold calcium spike (LTS) bursts occur only after thalamic neurons have been hyperpolarized. Since the RT provide major inhibitory inputs to thalamic nuclei, RT may play a key role in regulating switch between tonic and burst firing of thalamic neurons, and consequently regulate nociceptive signal processing. This study attempted to study the role of RT in nociception processing by recording RT neuronal activity changes to formalin injection in awake behaving mice. Results showed that most neurons in the RT increased firing right after formalin injection while the rest decreased firing. Group of neurons that increased firing to formalin injection significantly increased firing rate right after formalin injection and over time firing rate gradually decreased. Overall, neurons in the RT responded to formalin, but their role in nociceptive signal processing is inconclusive at the moment.

Disclosures: Y. Huh: None. **J. Cho:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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The CH Foundation

Laura W. Bush Institute for Women's Health

Title: Lateralized effects of neuropeptide S (NPS) on amygdala output neurons in an arthritis pain rat model

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[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Behavioral, electrophysiological and biochemical data suggest right-hemispheric lateralization of amygdala functions in pain (Carrasquillo and Gereau 2007 J Neurosci, 2008 Mol Pain; Ji and Neugebauer 2009 J Neurophysiol; Gonçalves and Dickenson 2012 Eur J Neurosci). The neurobiological principle of hemispheric lateralization of amygdala function in pain remains to be determined. Neuropeptide S (NPS) was shown in our previous work (Ren et al 2013 J Neurophysiol; Medina et al 2014 Mol Pain) to increase feed-forward inhibition of neurons in the amygdala output region (central nucleus, CeA), resulting in the inhibition of pain-related behaviors. The aim of the present study was to determine potentially differential roles of NPS in pain-related activity of amygdala neurons in the left and right hemisphere. Extracellular single-unit recordings were made from CeA neurons in the left and right hemisphere of anesthetized adult male rats. Responses to brief (15 s) stimulation (compression) of the knee, background activity, and receptive field size were measured before and during stereotaxic application of an NPS receptor antagonist (SHA68) into the CeA by microdialysis. SHA68 increased the evoked responses of left and right CeA neurons under normal conditions. In the arthritis (kaolin/carrageenan) pain model, however, SHA68 significantly increased only the responses of CeA neurons in the left, but not right, hemisphere. These data suggest lateralized effects of neuropeptide S receptor activation in amygdala neurons in an arthritis pain model.

Disclosures: G. Ji: None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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MH085946

Lloyd M.Kozloff Fellowship

Discovery Fellowship

Title: Affective components of chronic pain reflect circuit-specific changes in the anterior cingulate cortex

Authors: *K. S. MEDA[1], T. PATEL[2], J. M. BRAZ[1], A. I. BASBAUM[1], V. S. SOHAL[2];
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Abstract: Aims: We aimed to identify a neural circuit that encodes affective salience of pain in the anterior cingulate cortex (ACC). The ACC is densely innervated by the mediodorsal thalamus (MD) and the basolateral amygdala (BLA). Although studies have focused on intrinsic changes in the ACC as the main driver of aberrant pain affect in chronic pain, the contribution of afferent information from the MD and BLA to the ACC has been largely unexplored. Here we tested the hypothesis that these long-range inputs to the ACC are physiologically altered in the setting of chronic pain, and that these changes contribute to changes in the behavioral manifestation of pain affect.
Methods: In two models of neuropathic pain- spared nerve injury and chemotherapy (Taxol) induced neuropathy, we used whole-cell patch clamp recordings in slice preparations of the ACC to identify changes in intrinsic properties of ACC pyramidal neurons, as well as in their response to stimulation of excitatory inputs from the MD and BLA. To stimulate these inputs selectively, we injected an AAV5-CaMKII-ChR2 into the MD or BLA. To inhibit these inputs selectively, an AAV5-Synapsin-Arch was injected into the same regions. The same optogenetic approach was used to stimulate or inhibit these projections *in vivo*, in a conditioned place preference paradigm.
Results: We found that layer V pyramidal cells of the ACC are hyperexcitable in the two models of neuropathic pain. We also recorded a significant reduction in the excitatory responses of ACC pyramidal neurons to optogenetic stimulation of MD inputs in nerve-injured or Taxol animals, compared to controls. In contrast, there was an increase in the excitatory responses of ACC pyramidal neurons to optogenetic stimulation of BLA inputs in the nerve-injured condition, compared to control mice. Behaviorally, we found that animals with chronic pain avoided contexts in which they received optogenetic stimulation of MD inputs to the ACC; control animals showed neither preference nor avoidance. Inhibiting MD inputs produced the opposite effect, i.e, preference in animals with chronic pain. Stimulating BLA inputs produced preference in both animals with chronic pain and controls, while inhibiting the same inputs produced aversion in animals with chronic pain only.
Conclusions: These findings demonstrate that nerve injury induces long-term physiological changes in a circuit containing the ACC, MD and BLA. It appears that these changes may be differentially implicated in the behavioral manifestation of pain affect. Thus, targeting particular components of this circuit may offer a novel therapeutic approach to treating maladaptive pain affect in chronic neuropathic pain.

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Poster

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Support: MRC Research Grant MR/M013901/1

Title: Characterizing the sensitivity of laser-evoked EEG potentials to stimulus intensity using signal detection analysis

Authors: *B. BECK[1], G. IANNETTI[2], P. HAGGARD[1];
[1]Inst. of Cognitive Neurosci., [2]Dept. of Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Laser-evoked potentials (LEPs) are considered an indirect measure of activity in afferent nociceptive pathways. However, they are also sensitive to factors that are not specific to nociception, such as stimulus novelty and saliency. We investigated the extent to which LEP components are sensitive to a sensory property of the nociceptive stimulus, namely, the stimulus intensity. We measured LEPs while participants discriminated between a higher level and a lower level of laser stimulation of A-delta afferents on the hand dorsum. A multiple linear regression method was used to automatically estimate single-trial amplitudes and latencies of the main LEP components (i.e., N1, N2, and P2). These measures were used to calculate neurometric measures of perceptual sensitivity (d') for each LEP component. A range of possible cutoffs in the amplitude or the latency of each component was used to classify each trial as either a neural "high" response or a neural "low" response. In a separate experimental session, EEG was recorded while the same participants discriminated between two levels of non-nociceptive transcutaneous electrical stimulation of A-beta afferents. The non-nociceptive somatosensory-evoked potentials (SEPs) from this condition were subjected to the same analysis, to address questions of nociceptive specificity. Preliminary findings suggest that the amplitude of the N2 LEP component contains information about stimulus intensity that is relatively independent of "bias" (i.e., the chosen amplitude cutoff for a "high response"). The equivalent N2 component of the non-nociceptive SEP does not exhibit the same sensitivity to stimulus intensity, despite having a signal-to-noise ratio similar to the laser-evoked N2. This suggests that the N2 component may contain more information about the intensity of nociceptive stimuli than the intensity of non-nociceptive somatosensory stimuli.

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Poster

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Title: Neural signatures of pain in animal models

Authors: *C. Y. SAAB, B. LEBLANC;
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Abstract: Pain is known to disrupt brain rhythms in humans. By contrast, little is known regarding the neural dynamics of spontaneous pain in awake animals. Here, we identify EEG signatures of pain and analgesia in rodents. Our data show increased power within the low frequency (3-10 Hz) range in primary somatosensory cortex (S1) and prefrontal cortex (PFC) in awake, freely-behaving rats with acute (intradermal capsaicin), inflammatory (intradermal CFA) or neuropathic pain (chronic constriction injury of the sciatic nerve). In the neuropathic pain model, coherence between PFC and S1 significantly increases at a late, but not early, time point during the development of nociceptive behavior. Treatment with ibuprofen, pregabalin or mexiletine at a clinically-relevant dose attenuates power and S1-PFC coherence. Our data suggest that cortical synchrony and cortico-cortical connectivity correlate with pain, analgesia and the transition from acute to chronic pain. We are currently investigating the thalamic origin of these neural signatures using a multi-channel recording system combined with optogenetic neuromodulation in awake mice.

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Poster

428. Pain: Thalamic and Cortical Processing

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Title: Anterior cingulate cortex (ACC) representation of aversive pain intensity

Authors: *Q. ZHANG[1], A. TONG[1], T. MANDERS[1], A. GARG[1], R. YANG[1], L. URIEN[1], Z. CHEN[2], J. WANG[1];
[1]Anesthesiol., [2]Psychiatry, New York Univ., New York, NY

Abstract: The anterior cingulate cortex (ACC) is well known to play a role in affective pain processing. It is less clear, however, if neural activities in the ACC can code aversive pain intensity. To address this question, we combine extracellular recordings in awake, behaving rats using tetrode arrays with optogenetic activation of the ACC during acute pain episodes. We use a laser to provide acute thermal pain of controlled intensity to the hindpaw. We also use a sharp 25g needle to provide acute mechanical pain and light touch as a negative control. With increasing laser intensity, rats demonstrate decreased paw withdrawal latency. Meanwhile, both spike rates and local field potentials (LFP) in the ACC respond to acute pain stimuli in a graded fashion. The percentage of neurons that increase their firing rates is inversely proportional to paw withdrawal latency. Furthermore, there is an increase in the LFP power at low frequencies with increasing pain intensity. Detailed analysis reveals that only a small fraction of the neurons in the ACC are truly pain-specific. We characterize such pain-specific neurons as cells that do not respond to non-nociceptive warmth or light touch, but increase their firing rates proportionally with increasing thermal pain intensity and also increase their firing rates to mechanical pain. Spike phase-locking analysis reveals that these neurons show significant phase synchrony to the LFP phase, indicating the role of timing code in response to transient pain stimuli with varying intensity. As LFPs originate from synchronous synaptic inputs into the local neural circuit, such phase synchrony suggests that a small fraction of pain-specific neurons communicate with local ACC neuronal population to provide pain-intensity coding. To further verify the role of ACC in pain coding, we perform decoding analysis using support vector machine (SVM) and K nearest neighbor classifiers (KNN). By selecting features with binned firing rate and low frequence LFP power, these machine-learning approaches can discriminate painful vs non-painful stimulus or low- vs high-intensity pain stimulus with high accuracy (up to 90%). Finally, we show that optogenetic activation of the ACC during the presentation of an acute pain stimulus increases conditioned place aversion (CPA) to that stimulus. Furthermore,

ACC activation during the presentation of a low-intensity pain stimulus has the same effect as a high-intensity stimulus. Together, our results indicate that the ACC plays a critical role in coding aversive pain intensity.

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Poster

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Title: Dorsal and ventral parts of the thalamic nucleus submedius provide two independent inputs to different areas of the rat orbitofrontal cortex: A single neuron-tracing study using virus vectors

Authors: *E. KURAMOTO[1], H. IWAI[1], A. YAMANAKA[1], S. OHNO[2], R. SENDO[2], K. KOYANAGI[3], S. TOYODOME[1], T. FURUTA[4], H. HIOKI[4], T. GOTO[1];
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Abstract: The orbitofrontal cortex is reported to have a role in various cognitive and behavioral functions, such as flexible behavior, outcome expectation, pain modulation, and fear conditioning. These orbitofrontal functions might be mediated not only by neuronal circuitry in the orbitofrontal cortex, but also by network between the orbitofrontal cortex and subcortical

structures, the striatum and thalamus. The thalamic nucleus submedius (Sm) is known to intensely project to the orbitofrontal cortex. The aim of this study is to define the underlying circuits connecting the Sm to the orbitofrontal cortex for the better understanding of the mechanisms of orbitofrontal functions. Thus, the axonal arborization of single Sm neurons was examined in rat brain using Sindbis virus vectors expressing membrane-targeted fluorescent proteins. First, the Sm was subdivided into dorsal part and ventral part: dorsal part was innervated by vesicular glutamate transporter 2-immunopositive afferents derived from the spinal trigeminal nucleus and spinal dorsal horn, but ventral part was not. We then visualized and reconstructed 5 dorsal Sm and 5 ventral Sm neurons at a single-cell level, and analyzed the difference of the axonal arborization between dorsal and ventral parts of the Sm. When the axons exited from the thalamus, the reconstructed Sm neurons always emitted axon collaterals to the thalamic reticular nucleus. In the cerebral cortex, both of the dorsal and ventral Sm neurons sent axons mainly to layers 2/3 and layer 5, and formed dense axon bushes. The spread of each axon bush was approximately 500 μm in diameter in the tangential direction to the cortical surface. Interestingly, the dorsal Sm neurons formed single axon bush restrictively in the ventrolateral orbital area, whereas ventral Sm neurons made 2 axon bushes in ventral, medial, and lateral orbital areas, as well as agranular insular areas, but not in ventrolateral orbital area. These results suggest that the dorsal and ventral Sm neurons play different roles in the orbitofrontal functions. The dorsal Sm might be able to activate neurons in ventrolateral orbital area and involved in pain modulation, because ventrolateral orbital area is known to modulate pain sensitivity through activation of a periaqueductal gray-brainstem descending pathway that inhibits the nociceptive inputs at the spinal and trigeminal dorsal horn. The ventral Sm neurons would be involved in fear conditioning by activation of neurons in ventral and medial orbital areas, and agranular insular areas, since these cortical areas are known to innervate the amygdala.

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Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Title: Dishabituation of central nervous system to tonic pain following chiropractic care - a standardized low resolution brain electromagnetic tomography (sLORETA) based study.

Authors: *M. NAVID[1,2,4], D. LELIC[1], I. NIAZI[4,5,3], K. HOLT[4], E. B. MARK[1,2], A. M. DREWES[1,2], H. HAAVIK[4];
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Abstract: It has been demonstrated that after chiropractic spinal manipulation neural plastic changes occur in different areas of the brain. Different methods have been utilized to assess these changes, but the majority of the measurements to find the involved brain areas have been indirect. The objective of this study was to determine the changes in brain activity during tonic pain after single session of chiropractic care in a sub-clinical pain population by using source localization of the EEG.
Fifteen healthy volunteers (10 males, 32.1 ± 7.2 years) participated in two experimental sessions on separate days; chiropractic or control (sham) session in random order. The EEG was recorded continuously using a 61-channel system before and after either intervention during 72s of cold pressor test at 2°C (left hand). The pain and unpleasantness ratings were obtained on two separate numeric scales (range: 0 = no unpleasantness/pain to 10 = maximum unpleasantness/pain). The EEG was divided into 9 epochs (8s each), which were separated into four frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-32 Hz). Subsequently, standardized low resolution brain electromagnetic tomography (sLORETA) was done on these frequency bands.
In the control experiment, the brain activity decreased in all frequency bands (all $p \leq 0.05$), whereas no change in activity was seen after the chiropractic session (all $p > 0.05$). The decrease in activity in the control arm was specifically seen in the limbic (delta), frontal (theta) and temporal (alpha and beta) lobes. There were no significant differences in pain and unpleasantness scores between pre and post-sessions in either of the two arms.
The decrease in brain activity in the control arm reflects central habituation which occurs due to repetitive painful stimulation. The lack of this phenomenon in the chiropractic arm could imply that the chiropractic care normalizes the central nervous system leading to central dishabituation following the session.

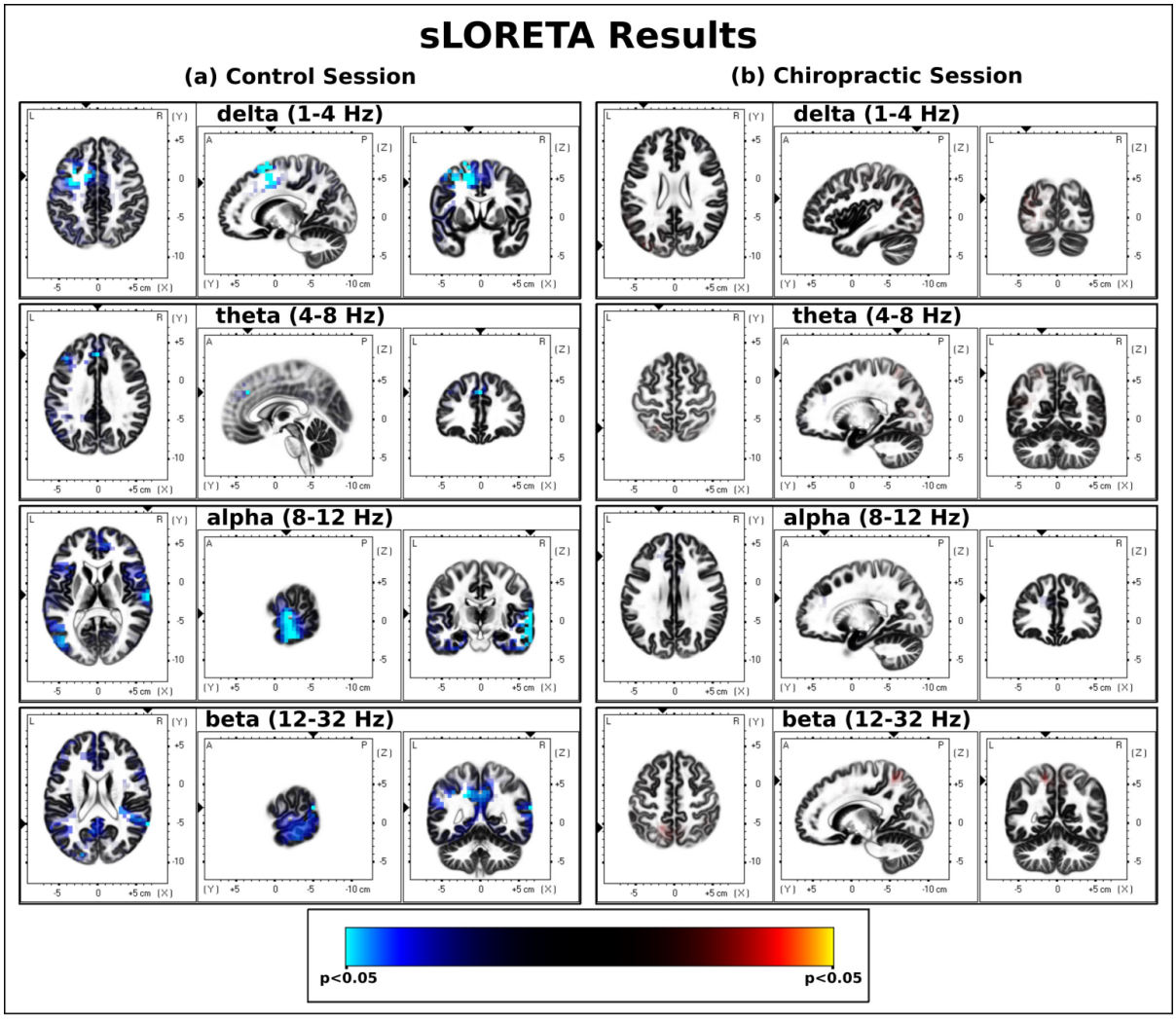

Figure: Slice views of source locations with the changes in activity for each frequency band after (a) control session and (b) chiropractic session compared to respective pre-session activity. Significant decrease in activity in all frequency bands can be seen in (a) (p≤0.05).

Disclosures: **M. Navid:** None. **D. Lelic:** None. **I. Niazi:** None. **K. Holt:** None. **E.B. Mark:** None. **A.M. Drewes:** None. **H. Haavik:** None.

Poster

428. Pain: Thalamic and Cortical Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 428.12/LL2

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS094389

Title: Effects of chronic pain on the cortical circuitry implicated in pain and endogenous analgesia

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Abstract: The central nervous system elicits endogenous analgesia via an extensive descending network. The periaqueductal gray (PAG), a key midbrain node within this network, is essential for suppressing ascending nociceptive signals and is a major pharmacological target of opioid based analgesics. The PAG receives significant input from the medial prefrontal cortex (mPFC) which is a cortical region that contributes to the affective and attentional components of pain. Functional and structural changes occur within the mPFC in human pain patients and in animal models of pain. However, pain-induced changes in defined mPFC neurons remain unclear. We hypothesized that cortico-PAG (CP) neurons would display altered local circuitry in the chronic constriction injury (CCI) model of neuropathic pain in mice. At post-operative day (POD) 7, a significant decrease in paw-withdrawal threshold (PWT) was observed for CCI animals compared to sham. At POD7, we targeted retrogradely labeled mPFC-CP neurons for local circuit mapping using glutamate uncaging in combination with laser scanning photostimulation (glu-LSPS). We found that the CCI resulted in significantly reduced local excitatory and local inhibitory inputs to CP neurons in both the prelimbic (PL) and inframbic (IL) regions of the mPFC. Although the input strengths were reduced throughout the cortical layers, inputs from layer 2 were significantly reduced in CCI CP-neurons. For the first time in a neuropathic pain model, we describe local circuit alterations to defined mPFC neurons within a key descending analgesic network. Our current efforts are focused on using optogenetics to dissect changes of long-range inputs to CP neurons in the CCI model. Collectively, circuit changes in the cortical control of PAG circuits could alter the homeostasis of the endogenous analgesic network and thereby diminish the intrinsic capability of the nervous system to suppress ascending nociceptive information.

Disclosures: J. Cheriyan: None. **P.L. Sheets:** None.

Poster

428. Pain: Thalamic and Cortical Processing

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS038261

NIH Grant NS081121

The CH Foundation

Laura W. Bush Institute for Women's Health

Title: SK channel function in CRF-containing amygdala neurons in a neuropathic pain rat model

Authors: *V. A. YAKHNITSA[1], T. KIRITOSHI[1], V. NEUGEBAUER[1,2];
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The central nucleus of amygdala (CeA) plays a key role in the regulation of the emotional-affective component of pain. The CeA is rich in neurons containing corticotropin releasing factor (CRF). Activation of CRF-CeA neurons and projecting pathways alters anxiety-like behavior and emotional responses. Our previous work showed that increased activity in CeA neurons in pain models drives emotional-effective responses and anxiety-like behaviors. Here we tested the hypothesis that dysfunction of small-conductance calcium-activated potassium (SK) channels contributes to increased tonic activity and excitability of CRF-CeA output neurons in rat model of chronic neuropathic pain. Whole-cell voltage- and current-clamp recordings were made from latero-capsular CeA neurons in brain slices from behaviorally tested normal/sham rats (controls) and neuropathic rats 3-4 weeks after spinal nerve ligation (SNL). Recorded cells were filled with biocytin and stained for co-localization with CRF. Neuropathic rats had developed mechanical hypersensitivity, increased vocalizations, and anxiety-like behavior by the time brain slices were obtained. CRF-positive CeA neurons recorded in brain slices from neuropathic rats lacked an apamin-sensitive medium afterhyperpolarization (mAHP) and showed increased excitability measured as the number of spikes in response to depolarizing current injections (F-I function) compared to CRH-positive neurons from control rats. The results suggest that loss of SK-channel function contributes to increased activation in amygdala CRH-positive output neurons mediating emotional responses in a neuropathic pain model.

Disclosures: V.A. Yakhnitsa: None. **T. Kiritoshi:** None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences KLMH2014ZG05

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Title: Brain-network mechanisms underlying the analgesic effect of electrical stimulation of periaqueductal gray

Authors: *N. WANG[1], Y.-L. SU[2], J.-Y. WANG[1], F. LUO[1];
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Abstract: Clinical studies have proved that deep brain stimulation (DBS) can produce therapeutic benefits for neurologic diseases. DBS has been employed as an important application to treat chronic intractable pain for many years. Periaqueductal gray (PAG) is a traditional stimulation target of DBS. Numbers of studies have proved the analgesia effect of PAG stimulation in animals with experimental pain and patients with chronic pain. However, the neural basis underlying PAG stimulation produced analgesia remains unclear, especially at the supraspinal level. In this study, neuronal activity within thalamocortical circuits in rats has been recorded using a multichannel single unit recording technique. Four brain regions in the lateral and medial pain systems have been focused in this study, including the primary somatosensory cortex (SI), anterior cingulate cortex (ACC), medial dorsal thalamus (MD), and ventral posterolateral thalamus (VPL). We attempted to explore the effects of ventrolateral PAG (vlPAG) stimulation on the discharge of single units as well as the functional connections between these regions. Some important findings emerged from this study: (1) vlPAG stimulation could inhibit the nociceptive behavioral responses of noxious thermal-induced acute pain. In the four recorded areas, vlPAG stimulation inhibited the nociceptive responses of single neurons by reducing the fraction of responding neurons and decreasing the neuronal response magnitude. The correlation between paw withdrawal latency and response magnitude was also inhibited by vlPAG stimulation. (2) Functional connection analysis revealed that vlPAG stimulation suppressed the pain-evoked changes in the information flow from ACC to MD and from SI to VPL. These results suggested that the inhibitory effect on both sensory and affective dimensions of pain may contribute to the analgesia effect of vlPAG stimulation on acute pain.

Disclosures: **N. Wang:** None. **Y. Su:** None. **J. Wang:** None. **F. Luo:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS038261

NIH Grant NS081121

The CH Foundation

Laura W. Bush Institute for Women's Health

Title: Prefrontal cortical feedforward inhibition of amygdala output neurons in arthritic and neuropathic pain rat models

Authors: *V. NEUGEBAUER[1,2], T. KIRITOSHI[1];
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. and Therapeut., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Hyperactivity in the amygdala output region (central nucleus, CeA) accounts for pain-related emotional responses and affective states. Direct excitatory inputs and feedforward inhibition modulate activity of CeA neurons. Feedforward inhibition is centered on a cluster of inhibitory neurons in the intercalated cell mass (ITC). Evidence suggests that the medial prefrontal cortex (mPFC) can modulate amygdala activity, but synaptic mechanisms and pain-related effects remain to be determined. Our previous work showed beneficial effects of restoring mPFC output on pain behaviors. Here we used optogenetics and patch-clamp in brain slices to determine mPFC-driven synaptic transmission onto CeA neurons and pain-related changes in this pathway. rAAV5/CaMKIIa-hChR2 (H134R)-eYFP vector was injected into the mPFC to express light sensitive channel rhodopsin 2 (ChR2) in mPFC pyramidal cells and their axons. Whole-cell patch clamp recordings were made from CeA neurons in brain slices from control rats and from arthritic (kaolin/carrageenan model) or neuropathic (spinal nerve ligation model) rats. Light activation (blue laser) of ChR2-expressing mPFC axons in the amygdala brain slice evoked glutamate-driven polysynaptic inhibitory postsynaptic currents (IPSCs) in CeA neurons; these IPSCs were blocked by bicuculline or NBQX. Largest IPSCs were usually evoked by light stimulation centered on the ITC area, suggesting activation of cortical fibers onto ITC cells. mPFC-driven feedforward inhibition was found under normal conditions and in the pain models. The data provide direct evidence for mPFC-driven feedforward inhibition of CeA neurons using optogenetics. Further, this control mechanism is available in pain conditions to inhibit amygdala output.

Disclosures: **V. Neugebauer:** None. **T. Kiritoshi:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS38261

NIH Grant NS081121

Title: Monomethyl fumarate (MMF) inhibits pain behaviors of arthritic rats: involvement of the amygdala

Authors: ***H. KIM**[1], J. M. THOMPSON[1], V. GANAPATHY[2], V. NEUGEBAUER[1,3]; [1]Pharmacol. and Neurosci., [2]Cell Biol. and Biochem., [3]Ctr. of Excellence for Translational Neurosci. and Therapeut., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Pain is the number one reason for patients to seek health care. To contribute to mitigating this major health care problem with significant emotional and affective components, we investigate novel and improved therapeutic interventions to restore or strengthen normal brain functions in pain models. Evidence suggests beneficial neuroprotective effects of fumarate-containing pharmaceuticals on cellular resistance to oxidative damage in central nervous system cells, potentially via up-regulation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. Dimethyl fumarate (DMF) has been clinically used to treat relapsing forms of multiple sclerosis and psoriasis, and it is rapidly hydrolyzed to monomethyl fumarate (MMF) in the body. However, the beneficial neuroprotective effects of DMF and its primary metabolite MMF in pain management remain to be determined. Hence, we examined the ability of MMF to inhibit emotional-affective pain behaviors through an action in the amygdala output region (central nucleus, CeA), which plays a key role in emotional-affective dimensions of pain and pain modulation. Systemic (intraperitoneal, i.p.) or stereotaxic (into CeA) administration of MMF inhibited audible (nocifensive response) and ultrasonic (averse affective response) vocalizations of adult male Sprague-Dawley rats 5 to 6 hours postinduction of arthritis in the left knee joint (kaolin-carrageenan model). Systemic, but not intra-amygdala, application of MMF also inhibited spinal reflexes significantly, increasing hind limb withdrawal thresholds. MMF had no effect in control rats. These data indicate that MMF can inhibit arthritis pain behaviors, and its effects on emotional-affective responses are mediated by an action in the amygdala (CeA).

Disclosures: **H. Kim:** None. **J.M. Thompson:** None. **V. Ganapathy:** None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Support: NIDCR grant DE022129

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Title: Vesicular Gamma-aminobutyric Acid transporter (VGAT) expression in the ventral posterior thalamus can modulate hypersensitivity following varicella zoster virus (VZV) infection of rat whisker pad

Authors: ***M. UMORIN**[1], C. STINSON[1], M. DENG[2], M. RAO[1], M. YEE[3], L. L. BELLINGER[1], P. KINCHINGTON[3], P. R. KRAMER[1];
[1]Biomed. Sci., Texas A&M Univ. Baylor Col. of Dent., Dallas, TX; [2]Oral and Maxillofacial Surgery, Wuhan Univ. Sch. and Hosp. of Stomatology, Wuhan, China; [3]Dept. of Ophthalmology and of Microbiology and Mol. Genetics,, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Gamma-Aminobutyric acid (GABA) stored with the help of VGAT in synaptic vesicles is one of the main inhibitory neurotransmitters found in mammals. The thalamus plays an important relay function in the central transmission of orofacial nociception. This study aimed at answering the question: can a decrease in VGAT expression in ventral posterior thalamus increase orofacial hypersensitivity following VZV infection in the whisker pad of male rats? Seven male Sprague-Dawley rats were injected into the right ventral posterior thalamic nucleus with adeno-associated virus containing VGAT shRNA or random shRNA construct under U6 promoter. One week later the left whisker pad was injected with either VZV-infected MeWo cell or non-infected cells. A week after the VZV injection the rats were tested with a place escape/avoidance paradigm test for the presence of nociception in the area around the injected whisker pad. After the second week of testing the rats were sacrificed and brain tissues were collected for immunohistochemistry of the thalamic tissue and VGAT expression analysis. Rats with VGAT expression inhibited by expression of shRNA exhibited increased and longer

orofacial hypersensitivity compared to the rats with normal VGAT expression. The results indicate that modulation of orofacial nociception signaling in the ventral posterior thalamus can occur through change in release of the inhibitory neurotransmitter GABA.

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Poster

428. Pain: Thalamic and Cortical Processing

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Program#/Poster#: 428.18/LL8

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS038261

NIH Grant NS081121

The CH Foundation

Laura W. Bush Institute for Women's Health

Title: Lateralized feedforward inhibition of amygdala output neurons in an arthritis pain rat model.

Authors: ***T. KIRITOSHI**[1], V. NEUGEBAUER[1,2];
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Synaptic plasticity and increased responsiveness of neurons in the amygdala output region (central nucleus, CeA) have been consistently found in acute and chronic pain models. Behavioral, electrophysiological and biochemical studies showed right-hemispheric lateralization of CeA function in pain models, but underlying mechanisms remain to be determined. In this study, we sought to determine potential differences in excitatory and inhibitory synaptic transmission onto the left and right CeA under normal conditions and in an arthritis pain model. Whole-cell patch clamp recordings were made from CeA neurons in brain slices from normal and arthritic rats (5-6 h after intraarticular injections of kaolin and carrageenan into the left or right knee). Slices from the left and right hemisphere were used and compared in each animal. Excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by electrical stimulation in the basolateral amygdala (BLA). Electrical stimulation of the BLA evoked monosynaptic EPSCs and polysynaptic IPSCs in CeA neurons. IPSCs were blocked by

bicuculline but were also sensitive to a non-NMDA receptor antagonist (NBQX), suggesting that they reflect glutamate-driven feedforward inhibition. Under normal condition, peak amplitude of IPSCs and the IPSC/EPSC ratio were larger in right than left CeA neurons. In the arthritis pain model, peak amplitude of IPSCs and the IPSC/EPSC ratio were decreased in right but increased in left CeA neurons. Importantly, the difference was independent of the side (left-right) of arthritis induction. The results suggest a baseline difference in the inhibitory control of CeA neurons between the left and right hemisphere, and a dramatic change in a pain model. Increased feedforward inhibition of the left CeA and loss of feedforward inhibition of the right CeA may account for pain-related hemispheric lateralization.

Disclosures: T. Kiritoshi: None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Support: NIH NS 085413

CDMRP PR 100060

Title: Hyperexcitability of somatosensory cortical neurons in CK1d migraine mutant mice.

Authors: *P. S. SURYAVANSHI[1,2], P. A. SAWANT-POKAM[2], K. C. BRENNAN[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Neurol., Univ. of Utah, Salt lake city, UT

Abstract: A mutation in casein kinase 1delta (CK1d) was identified in two families with advanced sleep phase syndrome and typical migraine with aura. Mice genetically engineered to express this mutation showed a reduced threshold for cortical spreading depression (a phenomenon that underlies both migraine aura and brain injury) and an increased sensitivity to nitroglycerine-induced mechanical and thermal hyperalgesia (both considered migraine relevant pain phenotypes). The cellular mechanisms underlying this network excitability are unclear. We examined the intrinsic and synaptic properties of layer 2/3 neurons of mouse somatosensory cortex, using *in vivo* and *in vitro* whole-cell current and voltage-clamp recordings in wild-type (WT) and CK1d mutant mice. *In vivo* intrinsic membrane properties were different in neurons of CK1d mice, showing a significant hyperpolarizing shift in resting membrane potential, and a trend towards increased input resistance compared to WT littermate neurons. Due to higher input resistance, CK1d neurons even with hyperpolarized Vm showed similar frequency of APs firing

(or slope of F/I slope) and rheobase. To characterize synaptic network-dependent activity, we recorded post-synaptic potentials in current clamp recordings *in vivo*. We observed decreased frequency, but increased duration, amplitude and rise slope of post-synaptic potentials in CK1d vs WT neurons. To determine the contribution of excitatory synaptic currents to these differences, neurons were voltage clamped at a holding potential of -70 mV, with a cesium-based internal solution *in vivo*. We found that excitatory currents in CK1d neurons had reduced frequency but increased amplitude compared to WT neurons, suggesting that changes in post-synaptic potentials were driven by excitation. We further characterized synaptic differences between CK1d and WT neurons in *in vitro* using voltage-clamp recordings. Consistent with our *in vivo* recordings, we observed a reduction in the frequency of AMPA-receptor mediated excitatory currents in CK1d, relative to WT neurons. In conclusion, we observed intrinsic and synaptic characteristics in CK1d neurons that may account for the excitable network phenotype: particularly an increased amplitude and duration of postsynaptic potentials, likely accounted for by increases in excitation. Hyperpolarized membrane potential and decreased frequency of events may represent an adaptation to chronic increases in synaptic excitability. As CK1d mice represent a transgenic model of *non*-hemiplegic migraine, these data may be helpful in elucidating mechanisms that are relevant to normal migraineurs.

Disclosures: **P.S. Suryavanshi:** None. **P.A. Sawant-Pokam:** None. **K.C. Brennan:** None.

Poster

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Support: NIH Grant NS038261

NIH Grant NS081121

The CH Foundation

Laura W. Bush Institute for Women's Health

Title: 5-HT_{2C}R blockade in the amygdala conveys analgesic efficacy to SSRIs in a neuropathic pain rat model

Authors: ***T. T. DANG**[1], G. JI[1], T. A. GREEN[3], V. NEUGEBAUER[1,2];
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Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [3]Pharmacol. & Toxicology, The Univ. of Texas Med. Br., Galveston, TX

Abstract: Neuropathic pain is a serious chronic condition associated with negative affective states, anxiety, and depression. Current treatments, including selective serotonin reuptake inhibitors (SSRIs), have shown only variable efficacy. Our hypothesis is that serotonin 5-HT_{2C} receptor (5-HT_{2C}R) in the amygdala accounts for the limited effectiveness of serotonin. The amygdala plays a critical role in the emotional-affective dimension of pain. 5-HT_{2C}R is widely distributed in the human and rat brain, particularly in the basolateral amygdala (BLA). A $G_{q/11}$ protein-coupled receptor, 5-HT_{2C}R has been found in GABAergic, glutamatergic, and dopaminergic neurons. 5-HT_{2C}R in the amygdala has been associated with anxiogenic effects. Here we tested the hypothesis that 5-HT_{2C}R blockage in the BLA improves the efficacy of an SSRI (fluvoxamine) in reducing emotional-affective behaviors in a neuropathic pain rat model (spinal nerve ligation, SNL). Spinal reflex thresholds, supraspinally organized audible (nocifensive response) and ultrasonic (emotional-affective response) vocalizations, elevated plus maze and open field test (EPM and OFT, anxiety-like behaviors), and immobility in the forced swim test (depression-like behavior) were measured in male Sprague Dawley rats, 4 weeks after ligation of the left L5 spinal nerve (SNL model). Behaviors were compared in rats with stereotaxic injection of 5-HT_{2C}R shRNA-AAV vector into the BLA for local knockdown of 5-HT_{2C}R and rats with stereotaxic control viral vector injection. The protocol was as follows. Hindlimb withdrawal thresholds and vocalizations were measured before and after administration of fluvoxamine (30 mg/kg, i.p.). Elevated plus maze, open field, and forced swim tests were performed the next day after another administration of fluvoxamine. Fluvoxamine increased reflex thresholds, decreased vocalizations, increased open arm preference (EPM), and increased center duration and decreased number of entries into the center (OFT) in neuropathic rats with 5-HT_{2C}R knockdown compared to control viral vector treated neuropathic rats. The results suggest that 5-HT_{2C}R knockdown can enhance the pain inhibiting effect of SSRIs.

Disclosures: **T.T. Dang:** None. **G. Ji:** None. **T.A. Green:** None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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NIH Grant NS081121

The CH Foundation

Laura W. Bush Institute for Women's Health

Title: SK channel-mediated electrophysiological and behavioral effects of riluzole in the amygdala in a neuropathic pain rat model

Authors: *J. M. THOMPSON[1], V. YAKHNITSA[1], G. JI[1], V. NEUGEBAUER[1,2];
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain, including neuropathic pain, is a prevalent and expensive healthcare issue, but in spite of its clinical importance, treatment remains a challenge. Therapeutic strategies are limited and characterized by variable efficacy and severe side effects. Complicating treatment further are emotional affective aspects of pain. The amygdala is a limbic brain region that plays a key role in mediating the emotional affective dimension of pain. Here we tested the beneficial effects of riluzole, a clinically available compound that can activate small conductance calcium activated potassium (SK) channels. We hypothesized that riluzole would inhibit pain-related changes in the amygdala output region (central nucleus, CeA), resulting in the inhibition of pain behaviors in a rat model of neuropathic pain (spinal nerve ligation, SNL). Whole cell current clamp recordings of regular firing CeA neurons were made in brain slices from control and SNL rats. Spinally organized hindlimb withdrawal reflex thresholds and supraspinally organized audible (nocifensive response) and ultrasonic (emotional affective response) vocalizations were measured in control and SNL rats. Measurements were made 4 weeks after left sided L5 spinal nerve ligation. CeA neurons in brain slices from SNL rats showed increased action potential frequency-current (F-I) relationship, which corresponded to increased vocalizations and mechanical hypersensitivity. Riluzole application inhibited neuronal firing, increased the SK channel mediated medium afterhyperpolarization (mAHP), and induced an inhibitory synaptic response to stimulation of the parabrachial input into the CeA in some but not all neurons. Systemic (i.p.) and stereotaxic (into CeA) application of riluzole inhibited vocalizations with little effect on withdrawal thresholds. However, these effects were variable and failed to return responses to normal levels. Inhibitory effects of riluzole on vocalizations were blocked by stereotaxic (into CeA) coapplication of an SK channel blocker (apamin), but not ACSF (control), indicating the contribution of SK channels to the effects of riluzole. The results suggest that riluzole has inconsistent electrophysiological and behavioral effects on SK channels in the CeA in a neuropathic pain model.

Disclosures: J.M. Thompson: None. **V. Yakhnitsa:** None. **G. Ji:** None. **V. Neugebauer:** None.

Poster

428. Pain: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation: Pain

Support: the Medical Research Center, 2012R1A5A2A44671346 from the National Research Foundation of Korea

Title: Neuronal encoding of modality and intensity of somatosensory stimuli in the mouse S1 cortex

Authors: *Y. KIM[1], C.-E. KIM[1], H. YOON[2], S. KIM[2], S. KIM[1];
[1]Dept. of Physiol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Kyunghee Univ. Col. of Korean medicine, Seoul, Korea, Republic of

Abstract: The function of the primary somatosensory (S1) cortex is to discriminate various somatic sensations including touch and pain. Unlike other sensations, such as vision, hearing, taste and smell, how such somatic sensations are encoded at the cellular level in the S1 cortex is poorly understood. Using *in vivo* two-photon calcium imaging, we investigated the tuning profiles of layer 2-3 neurons in the mouse S1 cortex in response to distinct modality (i.e. innocuous press vs. noxious pinch) and intensity (i.e. different pinch intensity) of somatosensory stimuli. We found that S1 cortex neurons show mixed selectivity for different stimulation modality with co-existence of wide dynamic range neurons and low/high threshold cells. In contrast, a majority of neurons showed the similar response properties to different intensities of pinch stimulation while selective responses were observed only in a small subset of neurons. These results suggest that S1 cortex neurons process modality and intensity of somatosensory stimuli using differential encoding strategies.

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Poster

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Topic: D.02. Somatosensation: Pain

Title: Short-term effect of transcranial direct current stimulation (tdcs) in healthy subjects: somatosensory and pain threshold

Authors: *M.-S. HUNG[1,2];
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Abstract: Background: Primary motor cortex hand (M1) area is the common site to set as tDCS stimulate target. It can alleviate pain in patients who suffered from neuropathic pain. But rare investigate can demonstrate the sensory and pain modulate of tDCS. We seek to test how the single-session anodal tDCS alleviate acute pain immediately in healthy subject. **Hypothesis**: Single-session tDCS stimulation can (1) modulation of the pain threshold (2) reduce the grade of moderate pain stimulus before tDCS stimulus and (3) it also reflected in cerebral activity as measured with evoked potentials (EPs). **Materials and Methods**: There have 48 healthy subjects (Female:Male=1:1) attended the experiment. Each one receive once 20 minutes or 30s tDCS stimulation. Somatosensory and pain threshold (including electrical and heat stimulus) were recorded before and after tDCS stimulus. Contact heat evoked potentials (CHEPs) for the moderate pain temperature also were recorded before and after tDCS sessions. **Results**: Single session anodal tDCS over M1 can significant elevated electrical and thermal pain threshold but not sensory threshold. It means for (1) anodal tDCS has improved short-term effect even if on normal neural activity, (2) Once tDCS session used in M1-F4 montage can modulate pain threshold independently, or pain threshold were easier to modulated then sensory thresholds. **Conclusions**: There has no side effect in all subjects. In healthy subjects, once 20minutes anodal M1 tDCS stimulus can alleviate pain threshold (including electrical and heat pain) but the sensory threshold still stable. The mechanisms of pain reduce might not due to sensory threshold modulate. The short-term pain alleviate effect is effectively on healthy subject, it worth to investigate the long-lasting pain reduce effect.

Fig. In daily ratio changed, independent T test was be used for ESST, ESPT, HST and HPT. There has significant difference on ESPT and HPT ($p<.05$).

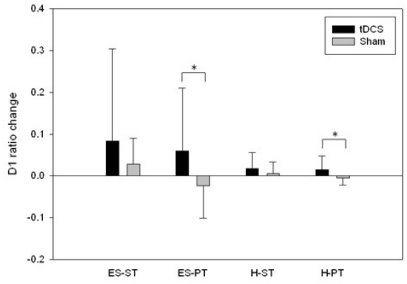
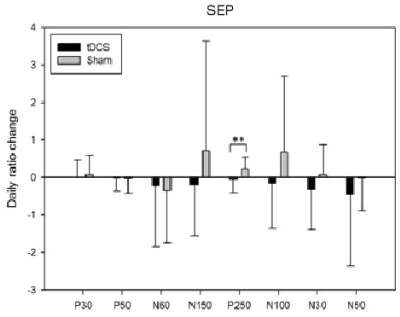


Fig. Daily ratio change in SEP doesn't with significant difference expect CZ-P250 ($p=0.01$)



Disclosures: **M. Hung:** None.

Poster

429. Somatosensation: Thalamocortical Processes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 429.01/MM6

Topic: D.03. Somatosensation: Touch

Support: NSF-GRFP

DGE-1058262

R01-NS050434

Title: The neurochemistry of thalamic reticular cells and its relationship with cell physiology and synaptic connectivity

Authors: R. MARTINEZ-GARCIA, B. VOELCKER, S. L. PATRICK, B. W. CONNORS, *S. J. CRUIKSHANK;
Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Neurons of the thalamic reticular nucleus (TRN) are the main source of inhibition in the thalamus. These GABAergic cells receive input from both thalamic and cortical neurons and are critical nodes in thalamocortical processing. TRN cells are thought to mediate synchronized activity during sleep and some types of seizures, and may be critical for selective attention. Previous studies have shown diversity in TRN cell physiology, morphology and synaptic connectivity, yet there is no generally accepted scheme for classifying TRN neurons. The neurochemistry of TRN cells may provide a basis for taxonomy, as it has for cortical interneurons. Whereas virtually all TRN cells seem to express the calcium binding protein parvalbumin (PV), we have observed that only subsets express calbindin (Calb1), or somatostatin (SOM), or both. Here we are testing whether TRN cell subtypes, defined by expression of these neurochemical markers, have distinct anatomical, functional, or synaptic properties in the somatosensory thalamus of mice. Anatomically, we observed that Calb1-expressing TRN cells were most concentrated in the central tier of the somatosensory sector of TRN, whereas SOM-expressing cells dominated the outer edges. Previous reports indicate that TRN cells projecting VPM thalamus vs. VPL/POM thalamus have a similar central vs. edge topography (Pinault, Br. Res. Rev., 2004). This suggests that neurochemically distinct TRN cell types differentially interact with the three main somatosensory relay nuclei, and may receive distinct cortical inputs as well. To investigate whether TRN subtypes differentially process synaptic inputs from thalamic or cortical sources, we are using optogenetics to control specific afferent populations while recording synaptic currents in pairs of neurochemically contrasting TRN cells (identified by fluorescent reporters in established knock-in lines). For example, we have used SOM-Cre x tdTomato mice to investigate synaptic input from corticothalamic cells onto SOM+ and SOM- TRN cells. We found that optogenetically activated corticothalamic inputs to SOM+ cells had lower thresholds than those to SOM- cells. Corresponding postsynaptic excitatory currents (EPSCs) were also larger in SOM+ than in SOM- cells for near-threshold stimuli. Curiously, the EPSC sizes in the two cell types converged at higher stimulus intensities. We are now studying inputs from thalamic relay nuclei, as well as the intrinsic cellular properties of TRN cells, to further characterize the relationship between neurochemical diversity of TRN and thalamic processing.

Disclosures: R. Martinez-Garcia: None. **B. Voelcker:** None. **S.L. Patrick:** None. **B.W. Connors:** None. **S.J. Cruikshank:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Topic: D.03. Somatosensation: Touch

Support: NIH Grant R01-NS050434 (to B.W.C.)

NIH grant F32-NSO84763 (to S.R.C.)

Title: Infrabarrels: Ensembles of structurally and functionally distinct neurons in layer 6a of mouse somatosensory cortex

Authors: *S. R. CRANDALL, S. L. PATRICK, S. J. CRUIKSHANK, B. W. CONNORS; Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Neurons of layer 6 (L6) are in a unique position to control the flow of information to and from the neocortex. The organization of L6 circuits is poorly understood, however. Here we used L6 corticothalamic (CT) specific Cre driver mice (Ntsr1-Cre) crossed to various reporter lines to study the circuit organization of L6a of the mouse primary somatosensory cortex, in particular the region representing the large mystacial vibrissae. Notably, we found that L6a contains discrete cytoarchitectonic units aligned with the barrels found in L4; we call these infragranular structures “infrabarrels”. Infrabarrels were not evident in L5 or L6b. Somata of excitatory CT pyramidal cells in L6a tended to cluster within infrabarrels, while non-CT excitatory neurons were densest within the septa between infrabarrels. The somata of parvalbumin- and somatostatin-expressing inhibitory interneurons were evenly distributed across L6a. Paired whole-cell recordings suggested that all CT and non-CT types of L6a excitatory neurons respond to ‘lemniscal’ input from the ventral posterior medial nucleus, with non-CT types receiving stronger input. In contrast, only non-CT types of L6a excitatory neurons responded to ‘paralemniscal’ input from the posterior medial nucleus of the thalamus. Our results show that the projection neurons of L6a are somatotopically organized into discrete ensembles, or infrabarrels. The structure and function of infrabarrel circuits will provide a foundation for understanding how parallel streams of sensory information are received and processed within the infragranular layers.

Disclosures: S.R. Crandall: None. **S.L. Patrick:** None. **S.J. Cruikshank:** None. **B.W. Connors:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Support: NINDPS Grant DP2NS087725-01

NEI Grant R01EY023756-01

New York Stem Cell Foundation Investigator Award

Title: Contextual modulation of spatial coding during active sensation

Authors: *E. LYALL[1], S. R. PLUTA[2], E. RYAPOLOVA-WEBB[3], G. I. TELIAN[3], D. E. TAYLOR[2], H. ADESNIK[2];
[2]Mol. and Cell Biol., [3]Helen Wills Neurosci. Inst., [1]Univ. of California Berkeley, Berkeley, CA

Abstract: Sensory context transforms how stimuli are represented in the neocortex, altering spatial representations, suppressing redundant information, and facilitating the encoding of higher order stimulus features. Despite the wealth of data on how sensory context influences neural computation under passive conditions, due to technical challenges, our understanding of the role of contextual modulation during active sensation is extremely limited. To address this gap, we probed how surround input influenced spatial coding in the rodent somatosensory system while animals actively explored space with their whiskers. By combining electrophysiological recordings and two photon imaging across four stages of the thalamocortical system, we found that surround whisker input imposes a dramatic transformation on how cortical neurons encode space. Unlike for thalamic neurons, surround suppression dominates L4 responses, and shifts spatial representations backwards in space. In layer 2/3 and layer 5 surround input provides a distributed mixture of facilitation and suppression that locally diversifies and globally distributes the cortical map of space. These data suggest that during active sensation contextual input increases spatiotemporal contrast in cortical population codes which may facilitate spatial localization and haptic perception.

Disclosures: **E. Lyall:** None. **S.R. Pluta:** None. **E. Ryapolova-Webb:** None. **G.I. Telian:** None. **D.E. Taylor:** None. **H. Adesnik:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Support: CIHR MOP-12675

CIHR FDN-143209

International Alliance of Translational Neuroscience

Title: Mapping cortical mesoscopic networks linked to the firing of single cortical and sub-cortical neurons

Authors: *D. XIAO[1], M. P. VANNI[1], C. MITELUT[1], A. CHAN[1], Y. XIE[1], A. CHEN[2], N. SWINDALE[3], T. H. MURPHY[1];
[1]Kinsmen Lab, Dept. of Psychiatry, Vancouver, BC, Canada; [2]Beijing Inst. for Brain Disorders, Capital Med. Univ., Beijing, China; [3]Dept. of Ophthalmology and Visual Sci., Vancouver, BC, Canada

Abstract: Understanding the basis of brain function requires knowledge of cortical operations over wide-spatial scales, but also within the context of single neurons. In vivo, simultaneous wide-field of view GCAMP imaging and sub-cortical/cortical cellular electrophysiology were used in mice to investigate relationships between spontaneous single neuron spiking and mesoscopic cortical activity using a spike-triggered mapping procedure. The procedure extends spike-triggered averaging methods to wide areas of cortex and provides selectivity by also employing genetically targeted indicators of neuronal activity. Spiking thalamic neurons was correlated with complex cortical spatiotemporal map features not predicted from consensus intra-cortical networks. Dynamically, single thalamic neurons predicted and reported specific cycles of wide-scale cortical inhibition/excitation. In contrast, spike-triggered maps from single cortical neurons tended to yield spatio-temporal maps expected for regional cortical consensus function. This approach can define network relationships between any point source of neuronal spiking and mesoscale cortical maps and may have use for identifying novel brain stimulation targets to affect connected areas.

Disclosures: **D. Xiao:** None. **M.P. Vanni:** None. **C. Mitelut:** None. **A. Chan:** None. **Y. Xie:** None. **A. Chen:** None. **N. Swindale:** None. **T.H. Murphy:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Topic: D.03. Somatosensation: Touch

Support: Boğaziçi University BAP project: 13XP8

Title: Modeling the vibrotactile responses of excitatory and inhibitory neurons in the hindpaw representation of rat SI cortex

Authors: *B. VARDAR, B. GÜÇLÜ;
Inst. of Biomed. Engin., Bogazici Univ., Istanbul, Turkey

Abstract: We have recently characterized the vibrotactile responses of regular-spiking (RS) and fast-spiking (FS) neurons in the hindpaw representation of rat SI cortex before and after bicuculline ($GABA_A$ receptor antagonist) microinjections. At all tested frequencies, the tonic responses of RS (excitatory) neurons increased, but the phasic responses did not change. Both phasic and tonic responses of FS (inhibitory) neurons were not influenced by bicuculline. We modified a computational model for whisker barrels (Pinto et al., 1996; J. Comput. Neurosci. 3, 247-264) to simulate vibrotactile responses of cortical neurons during bicuculline application. Specifically, our model included a thalamocortical input based on previous experimental data (de la Rocha and Parga, 2008; J. Comput. Neurosci. 25, 122-140), and generated by 5-Hz and 40-Hz sinusoidal bursts with 500-ms duration. Inhibitory weights on both excitatory and inhibitory populations were probabilistically decreased according to drug effects. In order to mimic the random variation among neurons, all weights were sampled from their particular probability distribution functions. We simulated firing rates of 14 RS and 11 FS neurons to compare with our experimental data recorded from the SI cortex. Percent changes in firing rate from the sham condition were calculated for both phasic responses (R_o: during the initial 100 ms of stimulus duration) and the tonic responses (R_d: during the entire stimulus duration of 500 ms). The results were analyzed by independent two-sample t-tests. For the 40-Hz stimulus, relative firing-rate changes due to bicuculline were very similar in simulation results compared to the experimental data (R_o: p=0.55 for RS, p=0.79 for FS; R_d: p=0.86 for RS, p=0.51 for FS). However, for the 5-Hz stimulus, the relative tonic firing-rate increase in the simulation results of RS neurons were significantly lower than the experimental data (R_o: p=0.28, R_d: p=0.006). On the other hand, the simulations were able to reproduce the experimental results of FS neurons for the 5-Hz stimulus (R_o: p=0.66, R_d: p=0.75). Overall, the modified computational model was successful for quantitatively simulating the relative excitatory/inhibitory interactions due to thalamacortical inputs driven by vibrotactile stimulation and the effects of bicuculline. It is important to note that the original model in the literature was established for mechanical ramp-and-hold stimuli on the

whiskers. The current work shows that our modified model still needs further improvement, especially at low frequencies, for incorporating vibrotactile stimuli applied on the skin.

Disclosures: **B. Vardar:** None. **B. Güçlü:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Program#/Poster#: 429.06/MM11

Topic: D.03. Somatosensation: Touch

Title: Pom thalamocortical input drives layer-specific response transformations

Authors: ***N. AUDETTE**[1], J. URBAN-CIECKO[1], M. MATSUSHITA[2], A. L. BARTH[1]; [1]Dept. Biol. Sci. and Ctr. for Neural Basis of Cognition, [2]Dept. Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Higher-order thalamic nuclei, such as the posterior-medial nucleus (Pom) in the somatosensory system or the pulvinar in the visual system, densely innervate the cortex and can influence perception and plasticity. To systematically evaluate how higher-order thalamic nuclei can drive cortical circuits, we optogenetically activated Pom axons in mouse primary somatosensory (barrel) cortex during genetically-targeted whole-cell recordings in acute brain slices. We find that ChR2-evoked thalamic input is differentially distributed across four major cell types in the neocortex, revealing layer-specific modules for the summation and processing of Pom input. Specifically, we find that direct Pom input to parvalbumin-expressing (PV) neurons is strong in L5a but absent in L2, where Pom activity drives firing in 5HT3a-expressing (5HT) cells. In both layers, direct synaptic input to somatostatin (SST) neurons was negligible. Evoked activity in pyramidal neurons from deep layers is fast and synchronized by rapid, PV-mediated feedforward inhibition, and activity in superficial layers is weaker and prolonged, facilitated by slow inhibition from 5HT neurons. A longer integration window for incoming sensory information in L2 may facilitate stimulus detection and plasticity over long time scales, requiring coincident inputs from multiple pathways to drive action potentials. Our data also recapitulate some cell-type-specific features of sensory-evoked activity – SST neuron hyperpolarization, and the initiation of recurrent network activity – that have been observed *in vivo*, indicating that Pom activity is sufficient to generate these phenomenon in acute brain slices with only local connections preserved.

Disclosures: **N. Audette:** None. **J. Urban-Ciecko:** None. **M. Matsushita:** None. **A.L. Barth:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Program#/Poster#: 429.07/MM12

Topic: D.03. Somatosensation: Touch

Support: NIH NS050437

Title: Corticothalamic neurons target fast-spiking and somatostatin containing interneurons with different short-term dynamics

Authors: *A. AGMON[1], H. HU[2];
[1]Dept Neurobiol & Anat, [2]Neurobio. and Anat., West Virginia Univ. Sch. of Med., Morgantown, WV

Abstract: The corticothalamic (CT) pathway is a massive feedback projection from pyramidal neurons in cortical layer 6 to the thalamus, in which CT axons make facilitating excitatory synapses on both thalamocortical relay neurons and reticular thalamic neurons. Corticothalamic neurons also emit intracortical collaterals which make excitatory synapses in layers 5/6 and in layer 4; however, data on the intracortical targets of these collaterals and on the short-term dynamics of these connections are inconclusive. To resolve these questions, we crossed the NtsR-Cre mouse line and the Ai32 Cre-responder line to express channelrhodopsin exclusively in CT neurons. Using thalamocortical slices from mouse somatosensory cortex, we performed dual whole-cell recordings from excitatory neurons (regular spiking, RS) or inhibitory interneurons (fast-spiking, FS or somatostatin-containing, SOM) in layers 4-6, while using blue light stimulation to depolarize CT axon terminals in the vicinity of the recorded neurons. Adding TTX and 4AP to the bath solution eliminated spiking and polysynaptic activity, revealing the direct postsynaptic targets of the CT neurons. We observed strong excitatory responses (EPSPs and EPSCs) in both FS and SOM interneurons in both layer 4 and layers 5/6, but only weak or no responses in Layer 4 RS neurons. To determine the short-term dynamics of these synapses, we recorded from connected CT-FS and CT-SOM pairs in layers 5/6. In response to 10-80 Hz trains of CT spikes, EPSPs in SOM interneurons facilitated, but those in FS interneurons underwent depression. We conclude that CT activation is likely to generate net inhibition in the cortical network; this inhibition will switch from proximally targeting to distally targeting as the firing frequency of CT neuron increases.

Disclosures: **A. Agmon:** None. **H. Hu:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Support: NIH Training Grant T90DA032466

NIH Grant R01NS085447

NSF Graduate Research Fellowship DGE-1148903

NIH NRSA Pre-doctoral Fellowship F31NS089412

Title: Understanding optogenetic stimulation strategies: a study of opsin-neuron models and their spiking behaviors

Authors: *A. WILLATS;
Georgia Inst. of Technol. & Emory Univ., Atlanta, GA

Abstract: Optogenetics is a powerful tool for eliciting a wide range of patterns of neural activity with genetic and anatomical precision. Paired with closed-loop stimulation, it is possible to produce desired dynamic firing rates with high precision and reliability. However, the choice of stimulus protocol greatly impacts neural response and careful design relies on understanding nonlinear, nonstationary response properties which depend strongly on neuron type. For example, thalamic neurons tend to burst in response to sharp increases of light, but this bursting can be alleviated by maintaining a constant DC-bias. By altering the stimuli along an axis from smoothly modulated, to sharp pulsatile stimuli, an experimenter can manipulate the proportion of burst responses in this neuron.In this work we develop the foundations of optogenetic stimulation waveform design by exploring algorithmic approaches to fitting mathematical models of spiking responses and characterizing the output behavior of model neuron types with distinct mathematical structures. The results of this investigation suggest practical design guidelines for stimulation waveform design that depend on the neuron response properties and the task goals, with a particular emphasis on closed-loop stimulation strategies for dynamically controlling firing rate.Specifically, we build a map from stimulus features and model types to resulting spiking behaviors. Our investigations explore stimuli which are informed by current experimental practice and include sinusoids, ramps, noise waveforms, and square pulses of light. Our preliminary work consists of predicting in-vivo extracellular responses to optogenetic stimulation in closed- and open-loop contexts. We demonstrate genetic algorithm approaches as a flexible tool for fitting diverse classes of models, including phenomenological (linear-nonlinear-poisson), biophysical (4-state opsin dynamics coupled to a leaky-integrate-and-fire-or-

burst model), and models of intermediate complexity (a simple opsin model coupled with an Izhikevich neurons). We quantify behaviors including fine and coarse temporal features. Given the importance of bursting in sensory pathways and the challenges it presents in controlling firing rate, we perform a detailed comparisonof bursting vs. tonic response properties.Our results provide practical algorithms for model fitting, a taxonomy of model neural response behaviors under different stimulation strategies, and guidelines for stimulation design under task-dependent criteria (e.g., synchronous responses, bursting behaviors, etc.).

Disclosures: A. Willats: None.

Poster

429. Somatosensation: Thalamocortical Processes

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NSF Graduate Research Fellowship DGE-1148903

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NIH NRSA Pre-doctoral Fellowship F31NS089412

Title: Closed loop optogenetic control of neural circuits *In vivo*: Developing design principles for controlling patterns of neural firing rate

Authors: *M. F. BOLUS[1], A. A. WILLATS[1], C. J. WHITMIRE[1], Z. COSTELLO[2], M. B. EGERSTEDT[2], C. J. ROZELL[1,2], G. B. STANLEY[1];
[1]Wallace H Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol. & Emory Univ., Atlanta, GA; [2]Sch. of Electrical & Computer Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: The rapid advancement of genetic tools has enabled control of neuronal activity at fast timescales in an anatomically targeted manner with cell type specificity. Until recently, an ‘open loop’ strategy in which stimuli are designed a priori has been the de facto method for optogenetic control of neural activity. However, this method is prone to error due to unaccounted for variability in evoked neural activity. In the face of these challenges, a better strategy is to ‘close the loop’ around the activity being controlled, using feedback to guide stimulation in real-time. Recently, our group demonstrated the use of closed loop optogenetic control (CLOC) to drive single unit firing to desired constant firing rates in vivo (Newman et al. 2015). In this paradigm,

extracellularly recorded spikes are isolated in the somatosensory thalamus of the anesthetized rat and sorted in real-time. Firing rate is estimated online using a fixed-bandwidth filter and compared to a target rate. The resulting error signal is filtered through a proportional-integral (PI) controller and used to continuously modulate optical stimulation of neurons expressing channelrhodopsin (ChR2). Using this approach, we have since demonstrated effective control of dynamic patterns of firing rate. To generalize these results to the control of arbitrary patterns of firing rates, a systematic method for designing closed loop optogenetic systems is required. To this end, we are developing design principles for controlling temporally dynamic patterns of firing rate while rejecting disturbances that are band-limited from 0-25 Hz, a frequency range relevant to active sensation in rodent somatosensory thalamus. Specifically, we focus on tuning parameters for firing rate estimation and control as a function of control task. To estimate instantaneous firing rate from spikes online, we employ an exponential filter, whose time constant determines the filter bandwidth. Computationally, we have found that the optimal filter time constant decreases nonlinearly as the frequency content in the control task increases. To design the controller, we implemented a linear-nonlinear-Poisson model which was sufficient to capture optogenetic response in certain operating regimes. In simulation, controller gains are tuned by maximizing closed loop performance on sinusoidal firing rate tasks, sweeping across frequencies. Tuned parameters are then tested experimentally. Principled CLOC design demonstrated here will enable precise control of rhythmic oscillations and will form the basic building block required for more complex spatiotemporal control of activity such as sensory representations in cortex.

Disclosures: **M.F. Bolus:** None. **A.A. Willats:** None. **C.J. Whitmire:** None. **Z. Costello:** None. **M.B. Egerstedt:** None. **C.J. Rozell:** None. **G.B. Stanley:** None.

Poster

429. Somatosensation: Thalamocortical Processes

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Topic: D.03. Somatosensation: Touch

Support: DFG Sta 431/11-1

Title: Intracortical network effects preserve thalamocortical input efficacy in a cortex without layers.

Authors: ***J. GUY**[1], A. SACHKOVA[1], M. MÖCK[1], M. WITTE[1], R. J. WAGENER[2], J. F. STAIGER[1];

[1]Univ. Med. Ctr., Goettingen, Germany; [2]Dept. of Basic Neurosciences, Univ. of Geneva, Geneva, Switzerland

Abstract: Layer IV of the rodent somatosensory cortex contains the somatotopic barrel field. Barrels receive much of the sensory input to the cortex through innervation by thalamocortical axons (TCAs) from the ventral posteromedial nucleus (VPM). In the *reeler* mouse, the absence of cortical layers results in the formation of mispositioned barrel-equivalent clusters of layer IV fated neurons. Although functional imaging suggests that sensory input activates the cortex in a correct somatotopic fashion, little is known about the cellular and synaptic properties of identified excitatory neurons of the *reeler* cortex. We examined the properties of thalamic input to spiny stellate (SpS) neurons in the *reeler* cortex with in vitro electrophysiology, optogenetics and subcellular channelrhodopsin-2 (ChR2) assisted circuit mapping (sCRACM). Our results indicate that *reeler* SpS neurons receive direct input from the VPM, in line with their well-documented function as a main class of thalamorecipient neuron. Thalamic input, however, was more spatially dispersed along the somatodendritic arbors of *reeler* SpS neurons, and was also weaker with respect to wild-type controls. In spite of its relative weakness, thalamic input reliably recruited network dynamics including recurrent excitation and feedforward inhibition. These results raise the possibility that an increase in cortical amplification, either through enhanced recurrent excitation or weaker feedforward inhibition, rescue a weakened thalamic input to the *reeler* cortex, thereby ensuring sensory input efficacy.

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Poster

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Fulbright Award

Brown Institute for Brain Science Graduate Research Award

Title: Neural dynamics of single units and local field potentials in mouse barrel cortex underlying detection at perceptual threshold

Authors: *H. SHIN, S. R. JONES, C. I. MOORE;
Brown Univ., Providence, RI

Abstract: Detection at perceptual threshold is a neocortex-dependent process. To study the neural dynamics underlying successful sensory processing, we employed a well-controlled vibrissae deflection detection task in mice. Here we describe preliminary results from large-scale chronic electrophysiology recordings in the barrel cortex, the vibrissae region of the primary sensory area of the neocortex (2 mice, 34 sessions of high psychometric performance). Consistent with prior studies (Cauller & Kulics 1990; Jones et al., 2007), the somatosensory evoked potential showed a larger surface negativity (N1, thought to reflect feedback activity from higher sensory areas) at ~70ms after sensory stimulus onset on threshold-level hit (detected) trials compared to stimulus amplitude matched miss (non-detected) trials. Closer examination of the evoked potential further revealed a larger early surface positivity (P1, thought to reflect feedforward activity) at ~20ms on threshold level hits. Next, we characterized single and multi unit activity by quantifying the stimulus probability (SP, a measure of whether the unit was sensory driven; >0.5 if stimulus evoked, <0.5 if stimulus suppressed) and the detect probability (DP, a measure of whether the unit had differential activity in detected and non-detected trials; >0.5 if more activity on threshold level hits, <0.5 if more activity on threshold level misses). Well-isolated single units were classified into regular spiking (RS; n=76) and fast spiking (FS; n=69) units based on the spike waveform. Sensory-modulated RS units had a mixed distribution of sensory enhanced (4 out of 12 RS with SP significantly different from 0.5) and sensory suppressed units (8/12), whereas the majority of sensory-modulated FS units were excited by the sensory input (20/20). For both RS and FS units, DP during the evoked period (0~100ms, before the animals' reaction times) was positively correlated with SP during the same period, replicating previous studies (Yang et al., 2015). Interestingly, pre-stimulus DP and post-stimulus SP had a slightly negative trend in both populations. The majority of FS had hit-predictive post-stimulus DP (8 out of 9 FS with DP significantly different from 0.5 had DP significantly greater than 0.5), which manifested in the population average post-stimulus time histogram as higher evoked firing rate on hits than misses. For FS cells with hit predictive evoked DP, the activity on hits and misses started diverging at ~20ms, which coincides with the P1 of the evoked potential. This implies that feedforward excitation of barrel cortex FS neurons has an important role in perceptual modulation of detection.

Disclosures: H. Shin: None. **S.R. Jones:** None. **C.I. Moore:** None.

Poster

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Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 429.12/NN5

Topic: D.03. Somatosensation: Touch

Support: NIH MH106174

Title: Human beta rhythm modulation with perception and attention reflects the density of rhythmic transients across trials

Authors: *S. R. JONES[1], S. TSUTSUI[2], R. LAW[1], H. SHIN[1], C. I. MOORE[1];
[1]Dept. of Neurosci., [2]Brown Univ., Providence, RI

Abstract: Neocortical beta (15-29Hz) is one of the most prominent signatures of neural activity measured non-invasively in humans. Beta power is a strong predictor of healthy and pathological perceptual and motor performance. Yet, its precise causal role in function is unknown. Key to discovering beta's impact on function is a proper characterization the nature of beta in the un-averaged, unfiltered, time domain signals. We have previously shown that functionally relevant beta rhythms measured with magenetoencephalography (MEG) from primary somatosensory cortex (SI) and frontal cortex are transient in un-averaged data, emerging in high-power as brief events that last ~150ms. We have also shown that, on average, increased beta power predicted inattention and failed tactile detection (Jones et. al., J. Neurosci. 2010), and beta-band synchrony between SI and frontal cortex increased during inattention (Sacchet et. al., J. Neurosci. 2015). Here, we quantify beta in un-averaged data to uncover features that underlie the observed power differences with perception and attention across trials. We show that continuous bands of beta activity apparent in averaged data reflect the accumulation of discrete beta events across trials. Further, correlations between increased pre-stimulus beta power and decreased tactile perception and attention are driven primarily by an increase in the number of discrete beta "events" in the pre-stimulus period, rather than an increase in event amplitude or the duration of individual events. These results are consistent with other recent studies of local field potentials in monkeys that showed differences in averaged power across behavioral conditions reflect a change in the density of transients across trials (Feingold et. al., PNAS 2015, Lundqvist et. al., Neuron 2016), supporting the idea that this is a generalizable beta phenomena across tasks and species. In summary, our results suggest the transient nature of beta is an important feature to consider in developing models of beta's causal impact on function, and in designing stimulation paradigms aimed to modulate beta to improve function.

Disclosures: **S.R. Jones:** None. **S. Tsutsui:** None. **R. Law:** None. **H. Shin:** None. **C.I. Moore:** None.

Poster

429. Somatosensation: Thalamocortical Processes

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 429.13/NN6

Topic: D.03. Somatosensation: Touch

Support: NIH Grant MH106174

Title: Mechanisms of sensory inhibition induced by neocortical beta rhythms

Authors: *R. LAW[1], S. TSUTSUI[2], S. R. JONES[2];
[1]Neurosci., [2]Brown Univ., Providence, RI

Abstract: Beta frequency rhythms (15-30hz) are prominent throughout the brain and correlate with perception and attention as well as motor action. In human primary somatosensory cortex (SI), beta band magnetoencephalographic (MEG) spectral power in the prestimulus period inversely correlates with detection of a tactile stimulus (Jones et al 2010). Neural mechanisms that may link beta with perception, however, are not captured by contemporary models.
We have previously shown that beta in human SI is event-like, emerging for periods of ~150ms with a stereotypical waveform. Combining MEG, computational modeling and laminar recordings in animals, we also showed that beta can emerge from the integration of brief bouts of excitatory synaptic input to the proximal and distal dendrites of cortical pyramidal neurons, where the beta frequency is set by the duration (~50ms) of strong distal excitation.
Building on these results, we use a computational model of neocortical dynamics that accounts for the biophysics of MEG to study circuit mechanisms that connect beta rhythmicity to decreased tactile detection. We show that early tactile evoked potentials (<100ms) associated with high and low prestimulus beta power have distinguishable waveforms consistent with differences associated with detection. In a companion study (see Jones, Tsutsui, Law et al., SFN 2016), we also show that prestimulus spectral power differences with detection corresponds better to differences in the density of beta "events" than to differences in amplitude or duration of these events. Taken together, these results raise the possibility that beta's perceptual interference effect may result from the coincidence of an exogenously driven beta event with a tactile stimulus.
We model tactile stimuli occurring at several phases of a beta event as well as outside a beta event, finding that stimulation during a beta event can recruit a gamma cycle (~80Hz) in superficial layers, which inhibits the later (~70ms) network response that correlates with detection, generating current dipoles that qualitatively match early components of the event-related MEG signal. This effect depends on the phase of the underlying beta waveform. Our results provide a mechanistic link between beta rhythms and perception that may generalize to other sensory and motor processes.

Disclosures: **R. Law:** None. **S. Tsutsui:** None. **S.R. Jones:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 430.01/NN7

Topic: D.04. Olfaction and Taste

Title: Developing a bio-electronic nose by interfacing to the early olfactory system

Authors: E. SHOR[1], T. BOZZA[2], *D. RINBERG[1];
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Abstract: The mammalian olfaction sense is capable of detecting and classifying odors in a quick, reproducible and sensitive manner even in rapidly changing environment where many odorants are present and odor concentrations vary. This detection capability is superior to any electro-chemical detector technology present today. Consequently, animals are used to detect and sense odor response of various substances for a variety of healthcare, agriculture and homeland security tasks. However, although animals can detect and classify odors, we rely on behavioral training to allow those animals report which odors were detected. Here, we attempt to develop a bio-electronic nose by interfacing to the early olfactory system using high resolution ECoG array implanted on the olfactory bulb. By obtaining LFP recording in high spatial and temporal resolution of awake mice presented with various odors in different concentrations we develop a machine learning odor classification algorithm. The performance and sensitivity of this approach is compared to the sensitivity of other detection devices. This technology is also used to investigate the circuitry of the olfactory system.

Disclosures: **E. Shor:** None. **T. Bozza:** None. **D. Rinberg:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 430.02/NN8

Topic: D.04. Olfaction and Taste

Support: NIH R01 DC013802

Title: Onset latency analysis of odor-evoked calcium response in the juxtaglomerular cells of mouse olfactory bulb

Authors: *R. HOMMA[1], X. LV[2,3], S. ZENG[2,3], S. NAGAYAMA[1];
[1]Univ. of Texas Med. Sch. at Houston, Houston, TX; [2]Wuhan Natl. Lab. for Optoelectronics, [3]Dept. of Biomed. Engin., Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Olfactory glomeruli are the relay center where the principal neurons (i.e. mitral and tufted cells) receive the glutamatergic inputs from the olfactory sensory neurons. Different types of interneurons including both excitatory and inhibitory enclose each glomerulus and contribute to the odor information process within the glomerulus. These interneurons are collectively referred to as juxtaglomerular cells. Thanks to the past studies including slice preparation physiology and histology, major types of juxtaglomerular cells at least have been decently characterized. On the other hand, it is still far from clear how these neurons are coordinated to shape the glomerular inputs to the principal neurons. To fill this gap of understanding, it is important to look at ensemble activity patterns of population of juxtaglomerular cells that are associated with the same glomerulus. In this study, we used *in vivo* calcium imaging method to record odor response of multiple juxtaglomerular cells with Acousto-Optic Deflector (AOD) two-photon microscope. Anesthetized mice were used for this experiment. Neurons expressed a calcium indicator GCaMP6f through an AAV injection into the olfactory bulb preceding the optical recording. Odor-evoked calcium transients from 12 to 15 neurons surrounding a few glomeruli were recorded with a random-access scanning mode at a sampling rate of 667 to 833 Hz. We especially focused on the juxtaglomerular cells that were supposed to receive the inputs from the same glomerulus. These neurons were identified based on their odorant selectivity and the location of cell body (adjacent to the same glomerulus). Interestingly, neurons which presumably belong to the same glomerulus showed quite similar temporal patterns of calcium response. We carefully estimated the time at which odor-evoked calcium transient started rising from the baseline. We limited our analysis to the responses that are strong enough for accurately estimating the latency. Onset of odorant response of these neurons vary across stimulus-glomerulus pairs. Median across the cells within the glomerulus ranged from 18 to 235 ms after the estimated onset of first inhalation following stimulus presentation (25 stimulus-glomerulus pairs; mean 151 ms, SD 59 ms). The difference between the shortest and the longest latency within a trial was distributed between 10 and 79 ms (mean 33 ms, SD 15 ms). These distributions imply that the onset latency of juxtaglomerular neurons probably follow the time course of inputs from the olfactory sensory neurons and is less variable across the neurons within a glomerulus.

Disclosures: **R. Homma:** None. **X. Lv:** None. **S. Zeng:** None. **S. Nagayama:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.03/NN9

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC006441

NIH Grant DC012718

Title: Control of mitral/tufted cell output by selective inhibition among olfactory bulb glomeruli

Authors: ***M. WACHOWIAK**[1], T. BOZZA[3], K. R. HANSEN[2], M. N. ECONOMO[1];
[1]Dept. of Neurobio. and Anat., [2]Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT;
[3]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Inhibition is fundamental to information processing by neural circuits. In the olfactory bulb (OB), glomeruli are the functional units for odor information coding and contain multiple inhibitory circuits that shape glomerular output via mitral and tufted (MT) cells. How this inhibition is organized with respect to the glomerular map and how it manifests in response to odorant stimulation remains poorly characterized. Here, using ultrasensitive calcium reporters targeted to MT cells in combination with two-photon imaging from MT somata or apical tufts in awake and anesthetized mice, we were able to monitor odorant-evoked excitation and suppression of MT cell output. We found that MT cell response polarity was distributed across all MT cells innervating the same glomerulus, allowing us to relate patterns of MT excitation and suppression to the glomerular map. Odorants elicited unique patterns of excitation and suppression across glomeruli, and MT cells from any one glomerulus were excited or suppressed by different subsets of odorants. Excited and suppressed glomeruli were spatially intermingled, with both excitation and suppression sparsely distributed at low odorant concentrations and more densely distributed at higher concentrations. Using odorant mixtures, we found that this suppression is sufficiently strong to gate excitatory responses to other odorants and is mediated, at least in part, by interglomerular inhibitory interactions that differentially impact deep versus superficial MT cells. The specificity, concentration-dependence and spatial distribution of glomerular suppression are not easily explained by recently-proposed models of intensity-dependent feedforward inhibition or scaled global inhibition by inhibitory OB circuits. Instead, these results support a model of selective and nonrandom inhibition among glomerular ensembles and reveal that inhibitory OB circuits nonlinearly transform odor representations.

Disclosures: **M. Wachowiak:** None. **T. Bozza:** None. **K.R. Hansen:** None. **M.N. Economo:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: MEXT/JSPS KAKENHI Grant 15K06748

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Title: Structural basis for cholinergic regulation of neural circuits in the mouse olfactory bulb

Authors: M. HAMAMOTO, E. KIYOKAGE, *K. TOIDA;
Kawasaki Med. Sch., Kurashiki, Japan

Abstract: Odor information is regulated by olfactory inputs, bulbar interneurons, and centrifugal inputs from other brain regions. We have recently shown a unique distribution pattern of serotonergic neurons, one of the centrifugal inputs to the olfactory bulb. In this study, we focused on cholinergic regulation of the olfactory bulb (OB) and analyzed projection pathways of cholinergic neurons in detail. Cholinergic neurons in the nucleus of the horizontal limb of the diagonal band of Broca and the magnocellular preoptic nucleus project to the OB. Single cell imaging of a specific neuron within dense fibers is critical to evaluate the structure and function of the neural circuits. We labeled cholinergic neurons by infection with virus vector and then reconstructed them. We also examined the ultramicrostructure of synapses by electron microscopy tomography. To further clarify the function of cholinergic neurons, we performed confocal laser scanning microscopy to investigate whether other neurotransmitters are present within cholinergic axons in the OB. Our results show the first visualization of complete cholinergic neurons, including axons projecting to the OB, and also showed frequent axonal branching within the OB where it innervated multiple glomeruli in different areas. Furthermore, the synapse structure was found to be similar to that in serotonergic neurons. Although we have not yet detected the presence of other neurotransmitters, the diversity of synaptic morphology suggests multiple modes of transmission. Our present study elucidates the ways that cholinergic neurons could contribute to the elaborate mechanisms involved in olfactory processing in the OB.

Disclosures: M. Hamamoto: None. **E. Kiyokage:** None. **K. Toida:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH Grant MH101634-01

Brain and Behavior Foundation NARSAD Young Investigator Award

Title: Dynamics of neuronal ensembles in the main olfactory bulb

Authors: S. WADDLE[1], E. LYMAN[1], *K. PADMANABHAN[2];
[1]Univ. of Delaware, Newark, DE; [2]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Spontaneous activity across ensembles of neurons can reveal important features of network architecture, the responses of networks to inputs, and ultimately the encoding and decoding of sensory stimuli. In the olfactory system of the mammal, the main olfactory bulb is one of the first processing centers for sensory information. Complex interactions between the principle excitatory cells, the mitral and tufted cells, and local inhibitory neurons, govern the dynamics of activity, shaping how information is transmitted to higher processing centers. Understanding the dynamics of activity among these neurons, and the effect of those dynamics on olfactory coding remains an open question. Using high-density recordings in the main olfactory bulb, we characterized the dynamics of neuronal ensembles of mitral/tufted cells during epochs of sensory independent spontaneous activity. Complex interactions among populations of neurons were uncovered using maximum entropy models, providing important insight into structure of activity within the bulb. In addition to examining the features of activity across ensembles of neurons, we also developed a new approach to examine the effect that previous patterns of activity had on shaping future patterns of activity. Together, our work provides an important clue into how activity patterns in the bulb are evolved from the interactions between neurons, and how this activity is affected by the history of activity that preceded it.

Disclosures: S. Waddle: None. **E. Lyman:** None. **K. Padmanabhan:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH Grant DC012425

Title: Increased olfactory bulb BDNF does not enhance the normal survival of new granule cells and does not prevent deprivation-induced cell death

Authors: *K. M. GUTHRIE[1], R. BERGER[2], B. MCDOLE[2];
[1]Dept Biomed Sci., [2]Florida Atlantic Univ., Boca Raton, FL

Abstract: The adult subventricular zone (SVZ) harbors progenitor cells that give rise to new olfactory granule bulb granule cells throughout adulthood. Normally about half of these new cells survive to become functionally integrated into bulbar circuitry. A variety of molecular cues are known to regulate the survival of new granule cells in vivo, and sensory activity plays an important role as well. Olfactory deprivation by naris occlusion increases the number of new granule cells lost by apoptosis during a critical developmental window spanning ~14-21 days of cell age. The neurotrophin brain-derived neurotrophic factor (BDNF) exhibits activity-dependent expression and secretion in the CNS, and has well characterized effects on neuronal maturation and dendritic spine morphology during development and in adulthood. Augmenting BDNF availability in the adult rodent ventricular ependymal (VZ/SVZ) has been shown to promote the production and survival of adult-born granule cells in vivo. In contrast, studies employing knockout of the BDNF receptor TrkB in SVZ-born cells have reported no significant effect on granule cell survival. The aims of the present study were to determine 1) if increasing endogenous BDNF levels in the olfactory bulb, rather than the SVZ, enhances the survival of adult-born granule cells as they integrate into the granule cell layer (GCL), and 2) if increased BDNF can rescue new granule cells from sensory deprivation-induced apoptosis. We used adult transgenic mice that express increased BDNF throughout the olfactory bulb GCL. Elevated BDNF expression was confirmed by ELISA, and control and unilaterally-deprived mice were treated with bromodeoxyuridine (BrdU) to label dividing SVZ progenitor cells. Mice survived for 2, 4, or 8-9 weeks. Bulb sections were processed for co-localization of Brdu+/Neu+ to quantify numbers of new neurons in the GCL. Potential changes in SVZ cell proliferation were assessed by immunostaining for Ki-67, and levels of apoptotic cell death in the GCL were assessed with TUNEL labeling. In non-deprived mice, no significant differences in the numbers of BrdU+/Neu+ granule cells, Ki-67+ SVZ cells, or TUNEL+ bulb cells were found between transgenic mice and wild-type controls. Increased bulbar BDNF did not suppress apoptosis caused by sensory deprivation, or increase the number of new granule cells present in the

deprived bulb at 8 wks post-BrdU. Our findings support the view that while BDNF has significant morphological effects on granule cells (Bergami et al., 2013; McDole et al., 2015), its increased availability in the bulb has little impact on the survival of adult-born cells.

Disclosures: **K.M. Guthrie:** None. **R. Berger:** None. **B. McDole:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD R03DC013872

Title: Dynamic regulation of mitral cell spike synchronization and phase-locking by external tufted cells in a glomerular network model

Authors: ***C. RAPP**[1], F. FROHLICH[2], T. A. CLELAND[3], G. LI[2];
[2]Psychiatry, [1]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [3]Psychology, Cornell Univ., Ithaca, NY

Abstract: External tufted (ET) cells are glutamatergic interneurons within the glomerular layer of the olfactory bulb (OB) that recently have been shown to serve multiple important functions in the coding and processing of olfactory sensory information. ET cells possess striking dynamical characteristics such as spontaneous bursting at respiratory theta frequency, highly correlated activity, and burst entrainment to patterned olfactory input. By providing feedforward excitation to other juxtaglomerular interneurons and to principal output neurons (mitral cells), ET cells may regulate important OB computation such as signal amplification and glomerular synchronization. However, the functional roles of ET cells may critically depend on how olfactory information is transmitted to the mitral cells (MCs). In the traditional model, MCs are predominantly activated by olfactory sensory neurons (OSNs), while in a recent model, most or even all afferent excitation to MCs is delivered via intermediating ET cells. The computational implications of these two relay models in olfactory information processing remain unclear. To address this issue, we developed a biophysical model of the OB glomerular network consisting of OSNs, ETs, MCs and inhibitory periglomerular (PG) cells. The ET cell model contains multiple ionic currents as observed experimentally and accurately replicates the salient properties such as spontaneous bursting at theta frequency band and burst entrainment to olfactory input. The OSN is modeled as tonic spiking neuron and both MC and PG cell models are taken from previous study. We simulated both the traditional OSN-routing network and the ET-routing network under three

different sniffing frequencies (2, 4 and 8 Hz) and observed that MC spike synchronization and phase-locking to inhalation are dynamically regulated by ET bursting in the ET-routing network. When the sniffing frequency is low (e.g, 2 Hz), ET bursting is loosely entrained to the sniffing input, leading to weak MC synchronization and phase-locking. As the sniffing frequency increases, more ET bursting is entrained and the maximal entrainment is achieved at 8 Hz. Consequently, MC spike synchronization and phase-locking is the largest in response to 8 Hz sniffing input. In contrast, in the OSN-routing network, MC spike synchronization and phase-locking decreases with sniffing frequency. Thus, as sniffing frequency increases, the glomerular network may switch from an OSN-based computation to an ET-based computation that enhances the fidelity and precision of odor coding.

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Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: Novartis Research Foundation

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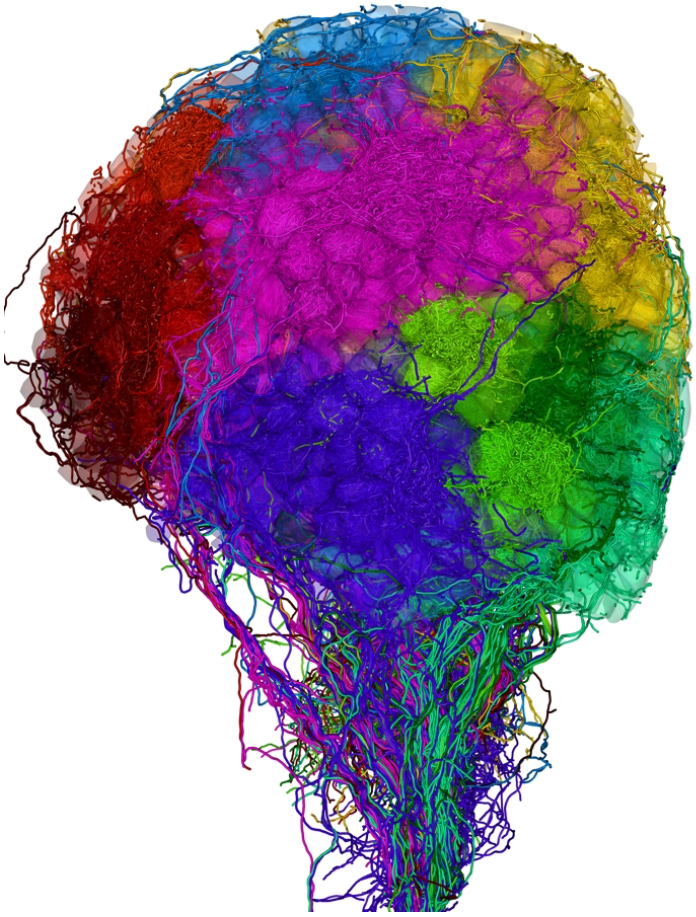
Title: Reconstruction of neuronal activity and connectivity patterns in the zebrafish olfactory bulb

Authors: *A. A. WANNER, C. GENOUD, R. W. FRIEDRICH;
Friedrich Miescher Inst. For Biol. Res., Basel, Switzerland

Abstract: In the olfactory bulb (OB), odors evoke distributed patterns of activity across glomeruli that are reorganized by networks of interneurons (INs). This reorganization results in multiple computations including a decorrelation of activity patterns across the output neurons,

the mitral cells (MCs). To understand the mechanistic basis of these computations it is essential to analyze the relationship between function and structure of the underlying circuit.
We combined *in vivo* twophoton calcium imaging with dense circuit reconstruction from complete serial block-face electron microscopy stacks of the larval zebrafish OB (4.5 dpf) with a voxel size of 9x9x25nm. We set up a high throughput neuron reconstruction pipeline with >30 professional tracers that is available for the scientific community (ariadne-service.com). To assure efficient and accurate circuit reconstruction, we developed PyKNOSSOS, a Python software for skeleton tracing and synapse annotation, and CORE, a skeleton consolidation procedure that combines redundant reconstruction with targeted expert input.
Using these procedures we reconstructed all neurons (>1000) in the larval OB. Unlike in the adult OB, INs were rare and appeared to represent specific subtypes, indicating that different sub-circuits develop sequentially. MCs were uniglomerular (see image) whereas inter-glomerular projections of INs were complex and biased towards groups of glomeruli that receive input from common types of sensory neurons. Hence, the IN network in the OB exhibits a topological organization that is governed by glomerular identity.
Calcium imaging revealed that the larval OB circuitry already decorrelates activity patterns evoked by similar odors. The comparison of inter-glomerular connectivity to the functional interactions between glomeruli indicates that pattern decorrelation depends on specific, non-random inter-glomerular IN projections. Hence, the topology of IN networks in the OB appears to be an important determinant of circuit function.



Disclosures: **A.A. Wanner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); European patent application EP14736451, US patent application US14897514, ariadne-service gmbh. **C. Genoud:** None. **R.W. Friedrich:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.09/OO1

Topic: D.04. Olfaction and Taste

Support: CRCNS: 1 R01 DC012943

Title: Mechanisms and functions of the offset response in insect olfaction

Authors: ***S. HANEY**[1], D. SAHA[2], B. RAMAN[2], M. BAZHENOV[3];
[1]Med., UCSD, Los Angeles, CA; [2]Washington Univ., St. Louis, MO; [3]Med., Univ. of California, San Diego, CA

Abstract: Insects respond to the olfactory stimuli with a strong transient response at odor onset followed by a moderate response during long odor presentations. Surprisingly, the neural response to the removal of olfactory stimulus, the offset response, is nearly as strong as the response to the application of olfactory stimulus, the onset response. We have previously found that this offset response: 1) governs stimulus termination behavior, 2) involves a disjoint (orthogonal) set of neurons from the onset response, and 3) is a common neural strategy to encode stimulus termination also employed in marmoset auditory cortex. However, little is known about the neuronal mechanisms of the offset response. What neural circuits generate the offset response? What effects might this have on the downstream neural targets? How might this impact the formation of memories? To address these questions, we have developed biophysically accurate computational model of the insect olfactory system. Based on the finding that odors can inhibit cognate receptors and depress firing rates of certain Olfactory Receptor Neurons (ORNs), we implemented odor-specific inhibition of a subpopulation of ORNs and synaptic adaptation at the ORN-AL synapse. We found that these two mechanisms are sufficient to produce offset responses that are a) orthogonal to the onset response and b) lacking in oscillatory behavior as seen *in vivo*. We next modeled the downstream Mushroom Body (MB) neurons, a region important in olfactory memory, and found that the onset and offset responses remain orthogonal in the high dimensional space of the MB neurons. MB neurons synapse with neurons in the beta lobe and these synapses are known to undergo spike timing dependent plasticity (STDP). Our study predicts that the offset response may contribute to meaningful and odor-specific synaptic

changes after multiple odor presentations. It remains, however, to be explored whether the odor offset activity may contribute to olfactory learning. The offset response exists in multiple different organisms and multiple different sensory systems but has gone largely unstudied. Our work presents a novel foundation for understanding this intriguing feature of sensory processing.

Disclosures: **S. Haney:** None. **D. Saha:** None. **B. Raman:** None. **M. Bazhenov:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.10/OO2

Topic: D.04. Olfaction and Taste

Title: Modulatory convergence of serotonin and dopamine in an olfactory network.

Authors: *K. M. LIZBINSKI, A. M. DACKS;
Biol., West Virginia Univ., Morgantown, WV

Abstract: Neuromodulation optimizes nervous system function by altering the synaptic efficacy and biophysical properties of individual neurons. The effects of neuromodulation are dictated by receptor expression patterns, receptor types and second messenger systems. Thus, the convergence of multiple neuromodulators on a single neuron involves the integration of several second messenger cascades, leading to potential non-linear effects on neuronal biophysical properties. Every neural network is influenced by a dynamic cocktail of neuromodulators that vary in their time course of action. For instance, serotonin (5-HT) and dopamine (DA) are both released by extrinsic neurons within the antennal lobe (AL) of the moth *Manduca sexta*, however they are associated with different physiological contexts. As a result, the extent to which they simultaneously affect olfactory processing is variable. Broadly, the integrated effects of multiple neuromodulators on sensory processing is not well understood. Here, we determined the consequences of integrating both 5-HT and DA on olfactory processing in the AL of *Manduca*. Using multichannel extracellular electrophysiology, we recorded the effects of 5-HT, DA and a combination of both modulators on odor-evoked responses of AL neural ensembles. DA and 5-HT affected overlapping populations of AL neurons, generally enhancing odor-evoked firing. However, each modulator had distinct effects on temporal structure of odor-evoked responses, with 5-HT extending response duration and DA shortening phases of post-excitatory inhibition. As a consequence the integrated effect of both modulators was overall excitatory, yet non-linear. Our data suggests that olfactory processing is contextually modulated based on a variable network state.

Disclosures: **K.M. Lizbinski:** None. **A.M. Dacks:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: DMS-1200004

Title: Integration of olfactory and mechanosensory stimuli in the projection neurons of antennal lobe in the moth Manduca sexta

Authors: ***H. LEI**[1], J. KIM[2], J. G. HILDEBRAND[2];
[1]Univ. Arizona, Tucson, AZ; [2]Univ. of Arizona, Tucson, AZ

Abstract: Olfactory receptor neurons (ORN) on the moth antennae sense environmental odors and transmit information to the interneruons, projection neuron (PN) and local neuron (LN), in the primary olfactory center, the antennal lobe (AL). The information about odors is synaptically processed within dense neuropil called glomerulus, which houses ORN terminals and dendrites of PNs and LNs, before sending out to higher olfactory centers in the protocerebrum. Additionally, mechanic stimuli, such as wind, are known to be an important sensory modality in moths' odor-seeking behaviors. Integration of mechanosensory and olfactory stimuli seems a beneficial feature, but where are these two modalities integrated? Our recent experiments suggest that the integration may occur as early as on the PNs. We recorded intracellularly from PNs' somata and stimulated moth antenna with odors carried by air puffs of different velocities, in addition to controls, i.e. air puffs alone. We found that increasing air velocity resulted in stronger responses to odors, and this phenomenon could not be explained by increased odor flux rate. While intermediate wind velocity caused significant increment of responses, both to odor and to control, higher velocity failed to enhance response further. It's still unknown what functional significance of mechanosensory responses in PNs might be, but we are currently testing a hypothesis that these responses may amplify odor signal. Supported by DMS-1200004 to HL.

Disclosures: **H. Lei:** None. **J. Kim:** None. **J.G. Hildebrand:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH R03 DC013997

Title: The connectivity of the serotonergic input to the olfactory system of *Drosophila*

Authors: *K. COATES, A. AUDA, C. MICHAEL, S. MICHAELS, T. SIZEMORE, A. MAJOT, A. DACKS;
Biol., West Virginia Univ., Morgantown, WV

Abstract: From one moment to the next, neuromodulators dynamically tune sensory processing to match the needs of the animal. By altering the biophysical and synaptic properties of individual neurons, neuromodulators alter the way that networks process information, which allows for adjustment of behavior. Neuromodulators strongly influence sensory processing, however the context in which neuromodulators are released within sensory networks is often complex. Modulatory nuclei are typically comprised of highly heterogeneous neurons that project to and receive input from many diverse brain regions, making it challenging to understand how modulatory systems affect sensory processing. Therefore, we sought to characterize the connectivity of two identified modulatory neurons in the olfactory system of *Drosophila melanogaster.* The contralaterally-projecting, serotonin-immunoreactive deutocerebral neurons, or CSDs, project broadly throughout the olfactory system, innervating both the ipsi-and contralateral antennal lobe, mushroom body calyx, superior lateral protocerebrum, and lateral horn. Moreover, they are the sole source of serotonin-immunoreactivity in the antennal lobe and lateral horn. Using a dendritic marker Dscam-GFP, we found that CSD neuron dendrites are found predominantly in higher order brain areas, including the antler and superior clamp. Using the axon marker synaptotagmin-GFP, we found that the CSD neurons have axon terminals in four olfactory processing regions, including the antennal lobe. Within the antennal lobe, the CSD neurons innervate the glomeruli to different extents. We therefore used the active zone marker Brp_{short} to quantify glomerulus specific differences in synaptic input from the CSDs. The density of active zones was consistent within identified glomeruli and lateral horn regions across animals. However, different glomeruli and lateral horn regions varied widely in CSD synaptic density. Taken together, our data suggest that the CSD neurons provided a unified source of modulatory input to several olfactory regions, yet their synaptic input may vary in an odotopic manner.

Disclosures: **K. Coates:** None. **A. Auda:** None. **C. Michael:** None. **S. Michaels:** None. **T. Sizemore:** None. **A. Majot:** None. **A. Dacks:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

Location: Halls B-H

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Program#/Poster#: 430.13/OO5

Topic: D.04. Olfaction and Taste

Title: The role of connectivity patterns in a computational model of *Drosophila* antennal lobe

Authors: *R. HAYNES[1], M. SAMAVAT[2], D. LULI[1], S. CROOK[1];
[1]Sch. of Mathematical and Statistical Sci., [2]Sch. of Electrical, Computer and Energy Engin., Arizona State Univ., Tempe, AZ

Abstract: In the olfactory system of *Drosophila melanogaster*, sensory transduction activates olfactory receptor neurons (ORNs) housed in the antennae and maxillary palps. These sensory neurons provide input to the antennal lobe, where the structural unit is the glomerulus. Each glomerulus is associated with at least three classes of neurons: excitatory local neurons (eLNs), inhibitory local neurons (iLNs), and projection neurons (PNs). PNs provide the output from the antennal lobe, sending axon terminals to the mushroom bodies and lateral horn, while the local neuron aborizations are confined to the antennal lobe.
In this work, we construct a conductance-based neuronal network model of the *Drosophila* antennal lobe, based on experimental data for *Drosophila* channel kinetics and constrained by specific odor stimulus/response characteristics described by Bhandawat et al. [1,2]. We use computational studies to investigate the role of interglomerular and intraglomerular connectivity patterns in the antennal lobe, where ORN inputs to the antennal lobe are modeled with Poisson spike trains, and model synaptic connections are mediated by chemical synapses and gap junctions as described in the *Drosophila* antennal lobe literature [3,4]. We find that in the case of homogeneous synaptic connection strengths, the model suggests that the eLN network facilitates odor detection in the presence of weak stimuli. Excitatory local neurons can spread excitation from PNs, amplifying weak inputs. However, depending on connectivity strength, excitatory LNs may decrease the ability of the network to discriminate between odors. In addition, computational studies suggest that gap junctions play a prominent role in facilitating synchronization of glomerular electrical activity. Various electrical connectivity regimes are explored to elucidate the role of gap junctions in the dynamical behavior of the antennal lobe network and the functional roles of different classes of neurons in odor detection and odor discrimination.
[1] Berger and Crook (2015) Frontiers in Computational Neuroscience 9:139.
[2] Bhandawat et al. (2007) Nat Neurosci 10(11):1474-82.
[3] Yaksi and Wilson (2010) Neuron 67(6):1034-47.
[4] Huang et al. (2010) Neuron 67(6):1021-33.

Disclosures: **R. Haynes:** None. **M. Samavat:** None. **D. Luli:** None. **S. Crook:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: NIH R03 DC013997

Title: Serotonergic modulation of inhibitory input to lateral horn modifies olfactory attraction in *Drosophila*

Authors: ***A. H. AUDA**, K. COATES, T. SIZEMORE, A. DACKS;
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Abstract: An organism must adjust its behavior based on the continuously changing environment. In response to the change in external conditions, network parameters are adjusted to generate different internal network states. These adjustments are often implemented via a variety of neuromodulators. Serotonin (5-HT) is widely distributed across the central nervous system and activates several receptor types. These receptors have different effects on biophysical and synaptic properties, thus allowing 5-HT to differentially target individual populations of neurons within a neural network. For instance, within the antennal lobe (AL), 5-HT enhances odor-evoked responses of projection neurons (PNs) and local interneurons (LNs), however the individual 5-HT receptors in the olfactory system responsible for the modulatory effects of 5-HT are not known. Using Drosophila, we have investigated the contribution of a specific 5-HT receptor expressed by a specific population of PN to the modulation of the lateral horn (LH), a second order olfactory network associated with processing innately important odor information. The ALs provide input to the LH via two types of PNs. We found that the GABAergic ventral projection neurons (vPNs) express the 5-HT1A receptor. Using GFP reconstitution across synaptic partners (GRASP), we found that the sole source of 5-HT to the AL and LH synapses onto vPNs in both regions, but not onto a subset of LH output neurons. To determine the functional role of the 5-HT1A receptor expressed in vPNs on odor-attraction, we reduced the expression of the 5-HT1A receptor in vPNs and tested olfactory sensitivity. The vPNs have been shown to respond to innately attractive odors and GABA release by vPNs in the LH is necessary for eliciting olfactory attraction. Consistent with these studies, we found that knocking down the inhibitory 5-HT1A receptor expression in the vPNs resulted in enhanced odor attraction. Therefore, our results suggest that 5-HT modulates innate odor-guided attraction by regulating the inhibitory tone exerted by the AL on the LH via the GABAergic vPNs.

Disclosures: **A.H. Auda:** None. **K. Coates:** None. **T. Sizemore:** None. **A. Dacks:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: NSF PHY-1058202

Ann M Hermundstad, Ph.D., holds a Career Award at the Scientific Interface from BWF.

Title: Disorder and compressive sensing in the olfactory system

Authors: ***K. KRISHNAMURTHY**[1], A. HERMUNDSTAD[2,3], T. MORA[4], A. WALCZAK[5], V. BALASUBRAMANIAN[2];
[2]Physics, [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Janelia Res. Campus, Ahsburn, VA; [4]Lab. de physique statistique, [5]Lab. de Physique Théorique, Ecole normale supérieure, Paris, France

Abstract: The olfactory system of animals is charged with identifying and discriminating familiar and novel odors which are complex mixtures drawn from a very high dimensional space of volatile molecules (perhaps 10^6 or more). Thus, the olfactory system must invest a limited number of genetically encoded receptor types (~50 in the fly, ~400 in humans, ~1500 in mice) wisely, to sense a much higher dimensional and highly variable signal. The responses are normalized (at the antennal lobe in insects and the olfactory bulb in mammals) and then projected, apparently randomly, to higher brain areas involved in learned behavior (mushroom body in insects and piriform cortex in mammals). We propose a new interpretation of the functional logic of this circuit pathway that is rooted in a key simplifying property of natural odors -- a typical odor is “sparse” in that it contains only 50-100 molecular components, a tiny fraction of the total number of volatile molecules. Drawing on the mathematics of random projections and on receptor response data, we demonstrate that the nervous system exploits the sparsity of natural odors by using disordered responses of olfactory receptors to create an efficient, distance-preserving embedding of the high-dimensional odor space in a low dimensional neural space. This efficient embedding is replicated by the subsequent random projections to the cortex, creating redundant and compact copies of the information that facilitate flexible learning. The theory makes striking new predictions, e.g. that arbitrary and small subsets of cortical neurons can provide complete representations of odor space to support complex animal behaviors. This prediction can be tested by optogenetic manipulation of the olfactory

cortex. In light of these results, we argue that the olfactory circuit pathway should be regarded as a novel scheme for compressive sensing of odors that developed through natural selection.

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Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: DFG-SFB 870

BayForIntAn

Australian Research Council future Fellowship

Title: Odor-evoked responses in both olfactory bulb and brain stem in a perfused preparation of the rat olfactory system

Authors: F. PEREZ DE LOS COBOS PALLARES[1,2], D. FARMER[3], D. STANIC[3], M. LUKAS[1], M. DUTSCHMANN[3], *V. EGGER[1];
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Abstract: To explore the mechanisms underlying network oscillations in the olfactory bulb (OB) and olfactory processing in the brain stem (BS), we adapted an established technique, the semi-intact *in situ* BS preparation, to develop a preparation with preserved anatomy of the olfactory system from the nasal cavities to the olfactory bulb and preserved respiratory circuitry. While the BS is oxygenated via the basilar artery, the OB is perfused in parallel via the ophthalmic artery. Although the forebrain is completely removed, the anatomical integrity of the nasal trigeminal afferents innervating the BS is not compromised.
In local field potential (LFP) recordings from the perfused OB, we observed robust spontaneous oscillations, mostly in the theta range. Odor application to the nasal epithelium with an olfactometer resulted in an increase in oscillatory power in higher frequency ranges, stimulus-locked LFPs, and excitation or inhibition of individual bulbar neurons that were established in multi-unit recordings, similar to odor responses reported from in vivo recordings.
Stimulation of the nose with room air caused weaker activation of the OB than odorant exposure. Moreover, irrigation of the nose with cold water did not evoke field potentials in the bulb but

triggered a breath hold signal in the simultaneous recordings of phrenic nerve activity due to the protective diving reflex. Moreover, such simultaneous recordings from the phrenic nerve and the OB also showed that odors can trigger significant and specific respiratory modulations via the trigeminal pathway. A non-trigeminal odor (rose oil) did not evoke significant respiratory modulation (n = 11 preparations), whereas trigeminal odors (menthol, lavender; n = 12) changed inspiratory activity in duration and amplitude in two phases: First, short inspiratory bursts were elicited in all recordings with parallel OB LFP responses, evocative of fictive sniffing. Second, specifically upon menthol application the respiratory frequency decreased compared to baseline activity. These changes were followed by an immediate recovery of the eupneic inspiratory activity after stimulation offset.
Since this preparation lacks cortico-limbic networks, our study demonstrates for the first time direct respiratory modulation via the trigeminal pathway in relation to odor sensing. We conclude that our method constitutes the first viable in situ preparation of a mammalian system that uses airborne odor stimuli, preserves characteristic features of odor processing and allows to to study odor-evoked signals within the olfactory bulb network and interactions between olfactory sensing and respiration.

Disclosures: F. Perez de los Cobos Pallares: None. **D. Farmer:** None. **D. Stanic:** None. **M. Lukas:** None. **M. Dutschmann:** None. **V. Egger:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: NIH Grant DC04285

Title: Assaying the spatial and temporal structure of olfactory bulb inhibition using paired recordings

Authors: *H. A. ARNSON, B. W. STROWBRIDGE;
Neurosciences, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: Inhibitory synaptic interactions play a central role in the processing of sensory information within the olfactory bulb. While the primary synaptic connections between principal cells and GABAergic interneurons, such as granule cells (GCs), have been described, less is known about the spatial and temporal scales over which these circuits operate. Previous work provides support for a wide potential range of spatial scales of inhibitory function mediated by

GCs. Individual GCs may influence principal cells spanning hundreds of microns if action potentials routinely drive GABA release throughout the entire dendritic arbor or may act in a spatially restricted manner (over several microns) if individual dendritic spines operate independently in response to highly localized sodium or calcium spikes. The spatial and temporal domains over which individual interneurons can functionally link principal mitral cells (MCs) and tufted cells (TCs) likely play a critical role governing how emergent network behaviors are generated during sensory processing.
In this study, we performed simultaneous intracellular recordings in acute rat olfactory bulb brain slices from MC - MC and MC - TC pairs to address the questions of temporal and spatial synchrony of GC activity. We found that ~40% of sampled pairs of MCs received low levels of coincident IPSCs greater than expected by chance as measured by the clipped cross-intensity function ($p<0.05$ threshold). Coincident IPSCs occurred in both proximal pairs and pairs separated by up to 350 μm, consistent with filtering properties of MC and TC lateral dendrites. In pairs of MCs separated by < 350 μm (and presumably sharing a common GC), we found little correlation between inhibitory connection probability and spatial scale. We also observed statistically significant synchronous activity in approximately 20% of MC - TC pairs separated by up to ~200 μm, suggesting that GCs functionally connect different sensory processing streams. The rate of synchronous activity is highly correlated to the baseline IPSC rate recorded in TTX, suggesting a reflection of intrinsic GC - MC circuitry. We find that IPSC synchrony occurs primarily over short time scales (< 2 ms) implicating coordination of GABA release sites by sodium-dependent spiking mechanism rather than through a slower, calcium-dependent mechanism. These results suggest that MCs and TCs appear to form random connections with GCs, enabling inhibitory postsynaptic responses to functionally link diverse subgroups of principal neurons during olfactory processing.

Disclosures: H.A. Arnson: None. **B.W. Strowbridge:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NSF Fellowship DGE-0925180

Title: Mimicking natural stimulation patterns to the olfactory bulb

Authors: *C. E. VAAGA, G. L. WESTBROOK;
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Abstract: Understanding how external stimuli are encoded in neuronal activity is a primary interest in sensory neurophysiology. In the olfactory bulb mitral cells and external tufted cells have unique physiological profiles to a single, brief afferent stimulus; however, their responses to afferent impulse patterns are less well understood. Odorant stimulation *in vivo* produces three distinct afferent impulse patterns: phasic, phasic-tonic and burst-pause-rebound (Savinger et al., 2009; Tan et al., 2010). Here, using single glomerulus stimulation in olfactory bulb slices, we examine the responses of principal neurons to "naturalistic" stimulation paradigms. Our stimulation protocol consisted of a transient phase that peaked at 100 spikes per sec and a tonic spiking rate of 60 spikes per second, which lasted for 0.5 seconds. In voltage clamp recordings, the monosynaptic afferent current in mitral and external tufted cells rapidly attenuated to the high stimulation frequencies, consistent with high release probability of afferent nerve terminals (Murphy et al., 2004). Furthermore, naturalistic stimulation resulted in long lasting depression of afferent responses in external tufted cells. In current clamp, mitral cells showed sustained firing despite the reduction in the monosynaptic response. Interestingly, the firing of mitral cells persisted well beyond the afferent stimulation, suggesting that these cells amplify afferent input. Conversely, the firing in external tufted cells followed the attenuation of the monosynaptic current, resulting in much briefer response profiles. Our results suggest that natural stimulation patterns result in different patterns of activation in mitral and external tufted cells, consistent with the view that these cells serve as unique, parallel input pathways with distinct function.

Disclosures: C.E. Vaaga: None. **G.L. Westbrook:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH Grant R15DC012425

Title: BDNF augmentation *In vivo* increases spine density in adult-born olfactory granule cells

Authors: *B. MCDOLE[1,2], C. ISGOR[2], K. GUTHRIE[2];
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Abstract: Brain-derived neurotrophic factor (BDNF) promotes the maturation, plasticity and maintenance of dendritic spines during development and in adulthood. In the main olfactory bulb, granule cells form a large population of inhibitory interneurons that regulate the activity of the bulb's excitatory projection neurons (mitral/tufted cells) through dendrodendritic synapses,

ultimately controlling the relay of sensory information to the primary olfactory cortex. Using Golgi staining, we previously have shown that over-expression of BDNF in the bulb granule cell layer increases spine density on granule cells throughout this layer. New granule cells are continuously generated from progenitor cells in the adult forebrain subventricular zone (SVZ). Knockout of the BDNF receptor TrkB in adult-born granule cells has been shown to impair their normal spine formation, implicating BDNF/TrkB signaling in the morphological development and integration of these new cells. The present study tested the hypothesis that spine development/maintenance in new granule cells can be promoted by increasing bulbar BDNF in vivo. Adult male transgenic mice over-expressing BDNF in the granule cell layer (TgBDNF mice), and their wild-type (WT) littermates, received SVZ-targeted injections of a lentivirus encoding a tau-mCherry fusion protein. In order to capture different stages of granule cell maturation, bulb tissue was collected at several time points post-infection. Using confocal microscope z-stack images, labeled granule cells were reconstructed in their entirety using Neurolucida software (MicroBrightfield Inc.). By 60 days post-infection, the latest time point examined, adult born-granule cells in TgBDNF mice exhibited a significant increase in apical dendritic spine density overall, as well as an increase the density of headed, mushroom-type spines, in comparison to WT controls. This included apical segments in the external plexiform layer (EPL) where dendrodendritic synapses with mitral/tufted cells are located. Additionally, estimates of GABAergic synapse number in the EPL, counted from immunolabeled confocal images, showed a significant increase in the density of gephyrin-positive puncta in TgBDNF mice relative to WT controls. Taken together with the evidence that TrkB knockdown impairs spine development in adult-born granule cells, these results provide insight into how BDNF/TrkB signaling contributes to the morphological integration of new granule cells by controlling spine maturation and maintenance.

Disclosures: **B. McDole:** None. **C. Isgor:** None. **K. Guthrie:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Human Frontier Science Program

Japan Science and Technology Agency(PRESTO)

Title: The role of olfactory bulb adult neurogenesis in olfactory representation and behavior

Authors: *W. L. LI[1], I. IMAYOSHI[3,4], T. KOMIYAMA[1,4,2];
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Abstract: Perceptual learning, a process that leads to improved ability to discriminate between and/or categorize similar sensory stimuli, is fundamental to complex cognitive processes and animal survival. Here we study mechanisms mediating olfactory perceptual learning in mice, focusing on the olfactory bulb(OB), where the initial olfactory information processing occurs. We have recently found that olfactory perceptual learning involves changes of principal neuron population dynamics in the OB, but the cause of these changes is unknown. In the adult OB, thousands of newly-born neurons arrive daily and differentiate into inhibitory interneurons. These adult-born neurons form extensive reciprocal connections with principal neurons and transiently display a higher level of plasticity. We hypothesize that they play an important role in mediating circuit plasticity underlying olfactory perceptual learning. Here we test this hypothesis using an inducible genetic method to ablate adult neurogenesis and testing their ability to discriminate between odors in a two alternative-choice discrimination task. We found that mice without adult-born neurons could discriminate two very distinct odors as accurately as control mice. However, in a task in which mice were required to categorize eight highly similar odors and respond accordingly, learning of animals with adult neurogenesis ablation was significantly slower. These results indicate that adult neurogenesis is important for olfactory perceptual learning. We are currently in the process of testing the hypothesis that these young adult-born neurons facilitate perceptual learning through modulating local principal neurons. Taking advantage of the chronic in vivo two photon imaging technology, we are recording the activity of principal neuron ensembles throughout the days of learning. Comparing the plasticity of principal neuron odor representations during learning between mice with and without adult-born neurons, we hope to shed light on the role of adult neurogenesis in perceptual learning and the underlying mechanism. Our preliminary data suggest that the discriminability of the principal neuron population is impaired in the absence of young adult-born neurons. We will present the latest results from these experiments.

Disclosures: W.L. Li: None. **I. Imayoshi:** None. **T. Komiyama:** None.

Poster

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Title: Synaptic distribution of individually labeled mitral cells in the external plexiform layer of the mouse olfactory bulb

Authors: T. MATSUNO, *E. KIYOKAGE, K. TOIDA;
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Abstract: Mitral cells are major projection neurons of the olfactory bulb. They receive olfactory inputs, regulate information, and send their axons to the olfactory cortex. To understand the regulation of the output of mitral cells, we established a method for visualization of individual projection neurons and examined the distribution of their synapses quantitatively. Individual mitral cells were labeled by viral injection, 3D-reconstructed with light microscopy and serial-sectioned for electron microscopy. Secondary dendrites of mitral and tufted cells were distributed within the inner and outer halves of the external plexiform layer respectively. Electron microscopically-reconstructed mitral cell bodies, proximal and distal secondary dendrites and primary dendrites were examined in approximately 100 to 370 serial thin sections each and their synaptic distributions were analyzed. The ratio of presynaptic sites (60%), reciprocal synapses (60% of presynaptic and 80% of postsynaptic sites) and neuronal profiles forming synapses with mitral cells and including reciprocal pairs (50%) were similar in each region. On the other hand, the density of synapses on the primary dendrites decreased with distance from the soma. The distributions of symmetrical synapses on mitral and tufted cells were also analyzed using synaptic and neuronal markers such as parvalbumin, vesicular gamma-aminobutyric acid transporter and gephyrin. Neurons immunoreactive for parvalbumin tended to form synapses on proximal secondary dendrites of mitral cells. In conclusion, we clarified the unique synaptic distributions on mitral and tufted cells and showed that local synaptic circuits of

these cells are restricted in the inner and outer halves of the external plexiform layer, respectively.

Disclosures: **T. Matsuno:** None. **E. Kiyokage:** None. **K. Toida:** None.

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The Francis Crick Institute

Title: Targeting dense reconstructions of an olfactory bulb circuit with X-ray and serial block-face electron microscopy.

Authors: ***C. BOSCH PIÑOL**[1], K. L. BRIGGMAN[2], M. HELMSTAEDTER[3], T. W. MARGRIE[1,4,5], A. T. SCHAEFER[1,4];
[1]Neurophysiol. of Behaviour Lab., The Francis Crick Inst., London, United Kingdom; [2]Circuit Dynamics and Connectivity Unit, NINDS, Bethesda, MD; [3]Dept. of Connectomics, Max Plank Inst. for Brain Res., Frankfurt, Germany; [4]Dept. of Neuroscience, Physiol. and Pharmacol., UCL, London, United Kingdom; [5]Sainsbury Wellcome Ctr. for Neural Circuits and Behaviour, London, United Kingdom

Abstract: The neuronal circuitry of the olfactory bulb glomerulus is the first site for sensory integration in the mammalian olfactory system. Across a mouse olfactory bulb approximately 1200 glomeruli are responsible for integration and transmission of the sensory input to projection neurons and thus olfactory cortex. Therefore understanding the details of both composition and connectivity of the glomerular circuit will be the basis of accurate models of early olfactory function. Here, we have sought to explore this question by performing serial block-face scanning electron microscopy (SBEM) analysis of a genetically defined glomerulus.
Automated EM volumetric approaches including serial block-face EM offer an outstanding platform to resolve these questions. Using a Merlin/3View SBEM we can acquire stacks of OB tissue with 32 nm slice thickness, 13 nm pixel size and 20 nm effective x-y resolution imaging with a 2 kV electron beam and current doses of 20 e^-/nm^2. Acquisition at these parameter sets requires 8 days per $(100\mu m)^3$ cube. While acquiring a high resolution stack from a glomerulus ($(180\ \mu m)^2 * 230\ \mu m$) together with a low resolution stack ($(500\ \mu m)^3$, 80 nm isotropic voxels,

imaging at 3 kV) through external plexiform and mitral cell layer is thus feasible within 72 days, this points to the need to target EM acquisition to the very region of interest as sampling larger areas for alignment at the EM level becomes prohibitively time consuming. A key challenge is thus to obtain focussed region-specific maps in a reproducible manner. Samples stained forSBEM typically become too dark to be aligned at the light microscope level; obtaining EM images on the other hand is associated with sample destruction. This points to a need for a volumetric technique that allows sample alignment and is compatible with EM-prepared tissue. By using correlative X-ray tomography - EM imaging we have been able to precisely target the high-resolution long-lasting acquisition of a specific olfactory bulb glomerulus at synapse resolution: The intensely heavy metal-loaded SBEM-prepared samples offer good contrast for imaging with a SkyScan 1172 microCT system. Therefore, the tomography contains 3D information of the histology of a large olfactory bulb sample (0.6 * 0.6 * 1.5 mm^3, at 5 μm resolution). This allows targeting a specific glomerulus, both narrowing the high-resolution acquisition in 3D and ensuring successful SBEM imaging from glomerular to mitral cell layer. This is the first step towards understanding the diversity of patterns of synaptic connectivity of the glomerular circuit and will pave the way for investigations into mechanisms of structural plasticity in this sensory system.

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Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: NIH grant ROI DC000566

Title: Control of the granule cell-short axon cell local circuit in the olfactory bulb by direct feedforward and feedback axonal inputs

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Abstract: GABAergic granule cells (GCs) in the olfactory bulb have been widely implicated in altering the activity of output mitral cells (MCs), for example in mediating lateral inhibition and synchronization. Recent studies have suggested that GC activity may itself be regulated by GABAergic deep short-axon cells (dSACs) located in the granule cell layer. We examined the

circuitry involved in modulating the GC-dSAC local circuit by performing whole-cell patch-clamp recordings in olfactory bulb slices from various mice, including two transgenic lines that express channelrhodopsin-2 (ChR2) in specific cell types. Light stimulation of tufted cells (TCs) in cholecystokinin-Cre-ChR2 mice elicited large monosynaptic excitatory post-synaptic currents (EPSCs) in both GCs (n = 6) and dSACs (n = 7). The rapid kinetics of the TC-to-GC EPSC together with the localization of the light pulse (around the GC soma) suggested that the feedforward excitatory signals originated from axon collaterals that terminate close to GC somata (Schoppa, 2006). Rapid monosynaptic EPSCs were also elicited in GCs (n = 22) and dSACs (n = 71) by stimulation of cortical feedback axons, either optogenetically in neurotensin receptor 1-Cre-ChR2 mice or via electrical stimulation in the anterior piriform cortex. If feedforward and feedback axons can directly excite both GCs and dSACs that inhibit GCs, we next wondered whether specific factors could alter the weighting of GC excitation versus inhibition. One such factor for the feedback pathway appeared to be cannabinoid (CB) receptors, as a 5-second conditioning depolarization of test dSACs resulted in a 36 ± 6 % (n = 41, p < 0.001) reduction of the feedback EPSC that was sensitive to the CB receptor type 1 (CB_1) antagonist SR 141716. Furthermore, the CB receptor agonist WIN 55,212-2 reduced feedback EPSCs in dSACs (by 62 ± 5 %, n = 5, p = 0.012), while also causing a transient increase (by 102.5 ± 27 %, n = 10) in the frequency of inhibitory PSCs in MCs evoked by feedback stimulation. Thus, by reducing feedback excitation of dSACs, activation of CB_1 receptors can favor excitation of GCs. Together, these results indicate that feedforward and feedback axons can directly excite both GCs and dSACs and that the net effect on GC-to-MC inhibition could depend on the depolarizing state of the network.

Disclosures: **F.R. Pouille:** None. **N.E. Schoppa:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 430.24/PP2

Topic: D.04. Olfaction and Taste

Support: DC014701

DC014367

Title: A model of experience-dependent odor construction in the olfactory bulb

Authors: ***A. BORTHAKUR**, T. A. CLELAND;
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Abstract: Reciprocal interactions between mitral and granule cells in the external plexiform layer (EPL) of the olfactory bulb transform the odor representation before transmitting the information to targets such as piriform cortex. However, the nature of this transformation remains unclear. Specifically, while the basic synaptic connections within the EPL are known, the topology of connectivity is not. Given that lateral inhibitory synaptic weights do not correlate with physical proximity (Fantana et al., 2008, Neuron 59:802-814), what then determines the distribution of synaptic weights among mitral cell principal neurons and inhibitory granule cells? We propose that the topology of connectivity arises from statistical learning based on olfactory experience. The question then is twofold. First, how does learning modify EPL topology in a way that contributes to odor perception and is consistent with existing data? Second, by what individual synaptic rules can the network generate the appropriate patterns of statistical learning? Current theory indicates that mitral cells can excite granule cells at any distance, but that granule cells can only effectively inhibit neighboring mitral cells (McTavish et al., 2012, Front Comput Neurosci 6:3; McIntyre and Cleland, 2016, J Neurophys), establishing some important topological constraints. Moreover, the principle that granule cells functionally inhibit mitral cell output by delaying their spikes rather than eliminating them adds to the accumulating evidence that odor representations at this level depend on fine-timescale spike timing differences. We began by assessing the effects of timing-dependent learning rules on mitral-to-granule synapses. We demonstrate that an asymmetric STDP rule generates higher-order receptive fields in granule cells that are more diagnostic for specific odors than are principal neurons. This constructive process enables olfactory bulb interneurons to learn patterns of covariance derived from the external environment. Critically, patterns of feedback inhibition mediated by granule cells can orthogonalize odor representations even for very similar odors, enabling very fine discrimination. The degree of discrimination is dependent upon the order of granule cell receptive fields, which are regulated by the maximum synaptic weight, synaptic connection probability, interneuron membrane time constant and spiking threshold, STDP parameters, and the amount of learning, in principle enabling the appropriate allocation of generalization and discrimination among specific odor representations.

Disclosures: A. Borthakur: None. **T.A. Cleland:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

Location: Halls B-H

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Program#/Poster#: 430.25/PP3

Topic: D.04. Olfaction and Taste

Support: NIH 1R01DC009948-01

NIH training grant 2T32GM007469

Title: Electrical stimulation of the locus coeruleus enhances signal to noise ratio in the olfactory bulb of anesthetized rats

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Abstract: Among other neuromodulatory inputs, the olfactory bulb (OB) receives abundant noradrenergic (NE) inputs from the locus coerulus (LC). Behaviorally, noradrenergic modulation in the OB has been shown to decrease odor detection thresholds and enhances odor discrimination at low concentration odors. It is hypothesized that NE does this in part through increasing signal to noise ratio of odor representations in the OB. We here test this hypothesis by electrically stimulating the LC in anesthetized rats to study how NE affects OB-dependent processing. We found that a majority of mitral cells show a significant modulation of spontaneous firing response to LC stimulation, with a subset of cells showing enhanced firing rates. In the presence of NE receptor antagonists, LC evoked modulation of firing rates is absent. The observed modulation of firing rate is strongly dependent on initial levels of spiking with low rates being increased and high rates decreased; the net result is a reduction in spread of spontaneous rates across mitral cells. Responses to odorants at three concentrations corresponding to vapor partial pressures of 0.01, 0.01 and 0.001 Pa were also modulated by LC stimulation. After LC stimulation, the number of significant excitatory responses to odors was somewhat increased, while inhibitory responses to odorants was decreased. Modulation of odor responses correlated strongly with that of spontaneous activity, suggesting that enhanced overall excitatory odor responses are due to the overall reduction of spontaneous firing rates, producing enhanced ability for excitatory responses to be seen, and inhibitory responses to be diminished. At the population level, neural representations across odor concentration were rendered more similar to each other by LC stimulation, suggesting an effect of concentration invariant representations. Similar results are shown by direct noradrenergic pharmacological manipulations within the olfactory bulb. These results together show that LC activity influences signal to noise ratio in the OB, and thus influences near threshold odor responsiveness.

Disclosures: L.C. Manella: None. **N. Petersen:** None. **C. Linster:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.26/PP4

Topic: D.04. Olfaction and Taste

Support: NIDCD R01 DC 00997701-06

Title: Middle tufted cell drive the mitral cell spatiotemporal firing patterns through glomerular and granule cell microcircuits

Authors: *F. CAVARRETTA[1,2], M. MIGLIORE[1,3], M. L. HINES[1], K. M. IGARASHI[4], G. M. SHEPHERD[1];
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Abstract: The olfactory bulb (OB) is a dual layer system, consisting of many glomerular units (GU) extending across the glomerular (GL) and the granule cell layers (GCL). The principal neurons are middle tufted (MT) and mitral cells (MC), whose activity encodes odor representation which is sent on the olfactory cortex (OC) through their axons. In turn, their activity is modulated by inter-glomerular lateral interactions which occur at both layers. Previously, our 3D biophysically and morphologically accurate OB models have included only MCs, GL, and GCL. It has been predicted that the GL microcircuits transform a dense and disorganized spatial glomerular activation, such as that exhibited by natural odors, into a sparse, normalized and contrast-enhanced one. Together, the GCL, over time, decreases the spatial representation overlaps of different odors after learning (Cavarretta et al., 2016). However, many questions remain open, especially regarding the role of MTs. In this work, we have implemented MTs in our 3D model. We show that both MTs and MCs contribute to the columnar organization of the GCL, in particular demonstrating that they must be inhibited by the same GL neurons, in order to generate learning-dependent columns (Migliore et al., 2007) which conform to those observed in the experiments (Willhite et al., 2006). The inclusion of MTs in the model also allowed an estimation of the relative proportion of the 3 subtype of granule cells (Woolf et al., 1991), for which there are no quantitative data. In addition, we found that MT membrane properties facilitate their precise inter-glomerular synchronization, which would be a key-feature to realize a fine temporal code relying on the inter-glomerular interactions in the GCL. Finally, assuming the MTs excite the deep short axon cells by their axon collaterals, we have inferred a plausible connection scheme in which the MTs could orchestrate the lateral inhibition produced by the GCL. These results provide insights into the role of the MTs in spatial and temporal odor coding, which will eventually lead to also include the OC in the 3D OB model, starting from those regions that are targeted from MCs and MTs axons and could act as a gate connecting the OB and OC, such as the anterior olfactory nucleus.

Disclosures: F. Cavarretta: None. **M. Migliore:** None. **M.L. Hines:** None. **K.M. Igarashi:** None. **G.M. Shepherd:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Topic: D.04. Olfaction and Taste

Support: FSU Chemical Senses Training (CTP) Grant Award T32 DC000044 from the National Institutes of Health (NIH/NIDCD)

Title: Possible roles for dopamine and vasoactive intestinal polypeptide in the circadian rhythms of the mammalian olfactory bulb

Authors: *K. S. KORSHUNOV[1], L. J. BLAKEMORE[2], P. Q. TROMBLEY[2];
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Abstract: Circadian rhythms influence nearly every daily biological process, from genetics to behavior, and are synchronized by the master circadian pacemaker, the suprachiasmatic nucleus (SCN). SCN ablation leads to the desynchronization of circadian rhythms of nearly every part of the body, with the exception of the retina and olfactory bulb (OB), which are proposed to maintain circadian rhythm synchrony due to their own circadian clocks. In the OB, circadian rhythms manifest in expression of certain genes, neuronal excitability, and odor sensitivity. However, the neuronal mechanisms that allow the OB to maintain independent circadian rhythms are not well understood. The OB's endogenous dopamine (DA) neurons play multiple roles in regulating OB circuit function and likely influence these rhythms, as DA neuron activity in the OB has been shown to vary with time of day as indicated by higher diurnal than nocturnal DA content and release. Also, DA is responsible for proper expression of circadian proteins in the retina, a structure that closely parallels the OB. In the SCN, where vasoactive intestinal polypeptide (VIP) is endogenous, VIP neurons synapse with hypothalamic DA neurons and regulate the circadian release of prolactin. This, as well as the finding that the VIP receptor, VPAC2, is important in synchronizing the expression of circadian genes in the OB, has led us to hypothesize that VIP regulates OB DA neurons. We have pilot data showing that VPAC2 is highly expressed in the glomerular layer of the OB and is co-localized with at least a subset of DA neurons. Additionally, we have found that VIP may affect the neuronal activity of DA and other OB neurons by influencing outward K^+ currents. We are currently working with a tyrosine hydroxylase green-fluorescent protein (TH-GFP) transgenic rat line to better target DA neurons for electrophysiological/pharmacological experiments so we can further establish the effects of VIP on the neuronal activity of DA neurons. Neuronal regulation of OB DA neurons by VIP may be one of the mechanisms that contribute to the endogenous circadian clock of the OB and to rhythmic olfactory functions.

Disclosures: K.S. Korshunov: None. **L.J. Blakemore:** None. **P.Q. Trombley:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.28/PP6

Topic: D.04. Olfaction and Taste

Support: ANR-12-JSV4-006-01

Title: Functional mapping of circuits mediating inhibition of olfactory bulb interneurons

Authors: *A. SANZ DÍEZ, N. BENITO, D. DE SAINT JAN;
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Abstract: Olfactory sensory neurons (OSN) carry odor information from the nasal epithelium to the olfactory bulb (OB) where it is transmited to Mitral and Tufted cells, the principal output neurons, within spherical structures called glomeruli. Each glomerulus is surrounded by periglomerular (PG) cells that mediate intraglomerular inhibition. PG cells have been classified as type 1 if they receive direct synaptic inputs from OSN terminals or type 2 if they do not. PG cells also receive inhibitory postsynaptic currents (IPSCs) but the circuits mediating this inhibition are unknown. We examined this question using whole-cell voltage-clamp recordings in mouse horizontal OB slices. Stimulation of OSN producing excitatory postsynaptic currents (EPSCs, holding potential Vh = -75mV) also evoked polysynaptic IPSCs in 6/11 type 1 PG cells (at Vh = 0mV). In contrast, OSN stimulation did not evoke IPSCs in type 2 PG cells (n=19), suggesting that inhibitory inputs onto type 2 PG cells are not generated by the glomerular network. However, distant stimulation within the glomerular layer (n=19) or in the internal plexiform layer (n=5) produced monosynaptic IPSCs in type 2 PG cells in the presence of NBQX and D-AP5 (amplitude 81.45 ± 44.92 pA; 20-80% rise time (RT) 0.77 ± 0.69 ms, decay time (DT) 17.23 ± 12.31 ms) with similar kinetics than spontaneous IPSCs. These results suggest that type 2 PG cells are inhibited by neurons with axons in deep layers and in the glomerular layer. Interestingly, we found that type 2 PG cells recorded in a transgenic mouse line expressing channelrhodopsin-2 (ChR2) under the control of the thy-1 promoter (Thy1-ChR2-YFP, Jackson Laboratory) responded with a TTX-sensitive monosynaptic IPSC to a short light pulse (490 nm, 1-10 ms) (n=17, amplitude 127.39 ± 151.38 pA (range 9-659 pA); RT 0.72 ± 0.34 ms and DT time 17.9 ± 28.16 ms, in the presence of NBQX and D-AP5). Light stimulation also evoked TTX-sensitive monosynaptic IPSCs in granule (n=5) and deep short axon cells (n=24) in this mouse. However, none of these OB interneurons (PG, dSA and granule cells) expressed ChR2 in Thy1-ChR2-YFP mice, suggesting that ChR2 is expressed in GABAergic centrifugal afferences projecting to the OB. Consistent with this idea, we have detected ChR2-YFP expression in GABAergic neurons of the Horizontal limb of the Diagonal Band of Broca (HDB), an area that sends dense GABAergic projections to the OB. HDB might thus be a major source of inhibition

of OB interneurons, an hypothesis that we are currently exploring using selective AAV-ChR2 transfection of HDB GABAergic neurons.

Disclosures: **A. Sanz Díez:** None. **N. Benito:** None. **D. De Saint Jan:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.29/PP7

Topic: D.04. Olfaction and Taste

Title: Reconstructing odor identity from olfactory bulb sequences using patterned optogenetics

Authors: *J. V. GILL[1,2], **J. M. KAPPEL**[2], E. CHONG[2], G. SERRANO[3], D. RINBERG[3]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Neurosci. Inst., New York Univ. Langone Med. Ctr., New York City, NY; [3]Neurosci. Inst., New York Langone Med. Ctr., New York City, NY

Abstract: In mammalian olfaction, the transmission of odor information relies on the activation of defined subsets of individual glomeruli located in the olfactory bulb. Odor cues are translated into complex patterns of glomerular activity and relayed to downstream regions in the cortex. To what extent these patterns contain odor information on a fine scale in space (single glomeruli) and time (milliseconds) is largely unknown due to limitations in manipulating glomerular activity. We used patterned optogenetics for instant generation and precise manipulation of glomerular activity to investigate spatiotemporal features of the glomerular code. Mice were trained to respond to 'artificial odors' composed of glomerular patterns generated with digital micromirror device (DMD)-driven optics. By observing the responses of mice to fine changes in patterns along spatial and temporal dimensions, we have identified features of glomerular activity that are critical to odor identity.

Disclosures: **J.V. Gill:** None. **J.M. Kappel:** None. **E. Chong:** None. **G. Serrano:** None. **D. Rinberg:** None.

Poster

430. Second-Order Processing of Olfactory Inputs

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Program#/Poster#: 430.30/PP8

Topic: D.04. Olfaction and Taste

Support: KRF Grant: WCI 2009-003

NIH Grant:DC005259

Title: Physiological and molecular phenotyping of glomerular layer interneurons in the mouse olfactory bulb

Authors: *O. R. BRAUBACH[1,2], T. TOMBAZ[3], T. GEILLER[1], R. HOMMA[4], T. BOZZA[5], L. B. COHEN[1,2], Y. CHOI[2,1];
[1]Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [2]Dept. of Cell. and Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT; [3]Dept. of Neurosci., Kavli Inst. for Systems Neurosci., Trondheim, Norway; [4]Dept. of Neurobio. and Anat., Univ. of Texas Med. Sch. at Houston, Houston, TX; [5]Dept. of Neurobio., Northwestern Univ., Evanston, IL

Abstract: Olfactory glomeruli are innervated by interneurons, which control glomerular excitability and cross-talk. The interneurons in the glomerular layer are very diverse and it has been difficult to understand exactly how they contribute to olfactory information processing. We are therefore studying the population activity of glomerular layer interneurons using different methods of olfactory and optogenetic stimulation. Our experiments were initially conducted in transgenic mice that express GFP in GABAergic (GAD65 and GAD67) and dopaminergic interneurons. These interneurons were bulk-loaded with AM calcium dyes, and their stimulus-evoked activities measured via acute *in vivo* two-photon calcium imaging. We determined that most interneurons responded preferentially to odor stimuli delivered at high concentrations; their numbers and signal sizes decreased as odors were presented at lower concentrations. Furthermore, interneuron responses also changed as odor stimulation lengths were varied. Specifically, short odor presentations (2-5 sec) elicited interneuron signals that were tightly coupled to the signals recorded from adjacent glomeruli, but prolonged odor exposures (20 sec) often triggered more diverse, decoupled cellular signals. We conducted similar experiments in mice that express channelrhodopsin in a single glomerulus (M72>S50-IRES-hChR-Venus). Optogenetic activation of this glomerulus at different intensities and durations replicated our findings from odor stimulation experiments, thus allowing us to map the interneuron population connected to a single glomerular unit. In a final set of experiments, we are combining calcium imaging with post-mortem immunocytochemistry targeted against the calcium-binding proteins calretinin, calbindin, S100 and neurocalcin. Consistent with previous results, we found that calcium binding proteins are expressed in subpopulations of GABAergic and dopaminergic

interneurons. We are now studying if molecularly distinct interneuron subpopulations comprise some of the physiologically identifiable interneuron pools that were identifiable based on their unique response patterns during various types of olfactory and optogenetic stimulation.

Disclosures: O.R. Braubach: None. **T. Tombaz:** None. **T. Geiller:** None. **R. Homma:** None. **T. Bozza:** None. **L.B. Cohen:** None. **Y. Choi:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 431.01/PP9

Topic: D.05. Audition

Support: NIDCD Grant DCO2514

Title: Independent attentional modulation of compound auditory feature integration and segregation

Authors: *K. N. O'CONNOR, A. J. PRABHU, J. S. JOHNSON, M. L. SUTTER;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: Attention plays a significant role in sensory detection. Although auditory scene analysis relies largely on the preattentive segregation and grouping of features based on their temporal and spectral attributes (Bregman 1990), attention also plays an important role in focusing on particular sound sources or streams (Cherry 1953). Auditory perceptual grouping and segregation depend on Gestalt-like rules (such as similarity and proximity along one or more dimensions), however little is known of attention's effects on perception in feature integration or segregation, when the differences between features are held constant. Intuitively it would seem that, the more readily that features may be segregated or independently processed, the greater their resistance to integration and grouping.
To address this question, we assessed human subjects' sensitivity for detecting one or two acoustic features presented simultaneously, using psychophysical methods. The features, broadband sinusoidally amplitude-modulated (AM) noise and bandwidth restricted noise (BWr), were chosen for minimal physical interaction. In an experiment designed to assess feature segregation, subjects were required to selectively attend to *either* AM or BWr during a session while ignoring the other feature. In the experiment designed to evaluate feature integration, subjects were required to detect a feature compound. Sensitivity in both cases was compared to single feature (SF) detection under either mixed (randomly interleaved trials) or blocked (SF only) sessions. A yes-no procedure was used: The subjects indicated using a joystick whether a

test stimulus was different than a previously presented standard stimulus (400-ms stimulus duration and interval). To date, three subjects have been tested in the selective attention (SA) experiment and two in the feature integration (FI) experiment.
Thresholds for feature detection were estimated from psychometric function fits using percentage of correct responses at seven levels of modulation /restriction, normalized for comparisons across condition. All subjects in the SA experiment were able to detect the attended feature with relatively low losses in accuracy (~0-20%), relative to SF stimuli, regardless of the magnitude of the unattended feature. However, both subjects in the FI experiment displayed relatively large drops in relative threshold (~10-50%) when detecting compound as compared to SF stimuli. These results support that, for relatively independent compound acoustic features, feature integration and segregation operate largely independently and are subject to attentional modulation.

Disclosures: K.N. O'Connor: None. **A.J. Prabhu:** None. **J.S. Johnson:** None. **M.L. Sutter:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

Location: Halls B-H

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Program#/Poster#: 431.02/PP10

Topic: D.05. Audition

Support: NIMH/DIRP

Title: Prefrontal and sensory correlates of auditory spatial attention in the macaque

Authors: *C. R. CAMALIER, A. BROWN, J. JACOBS, M. MISHKIN, B. B. AVERBECK; Lab. of Neuropsychology, NIH, Bethesda, MD

Abstract: Auditory spatial attention is critical for everyday interactions, especially hearing in noisy environments. In addition, attention is thought to be a largely “amodal” resource, but to test this assumption it is important to understand whether spatial attentional mechanisms differ across modalities (e.g. vision vs audition), particularly at the single-neuron level. Though fundamental to our understanding of attention and auditory processing, the neural correlates of spatial auditory attention remain poorly understood. This is due in part to the lack of a robust animal model, particularly one that shares key similarities with humans.
To address this, we have developed a novel spatial auditory selective attention task for macaques (n=2), based closely on human paradigms. In this task, a macaque monkey is cued to a particular side and must report the presence of a difficult-to-detect auditory target (embedded in noise) only

if it appears on the cued side. If it appears on the uncued side, he must ignore it. In this way, we are able to compare attention effects under identical auditory conditions.
We have begun collecting single neuron data from from two key areas implicated in the control of auditory spatial attention : prefrontal cortex (caudal principal sulcus; n = 163) and caudal auditory cortex (A1/CM/CL/TPT; n = 64) in one monkey during this task. In both areas, attention deployed to contralateral space significantly affected baseline firing rates in about 20% of the responsive neurons and also significantly affected 20-40% of responses to the masking noise. These early results are consistent with some of the hypothesized mechanisms reported during visuospatial attention, suggesting at least some attentional mechanisms are truly amodal.

Disclosures: C.R. Camalier: None. **A. Brown:** None. **J. Jacobs:** None. **M. Mishkin:** None. **B.B. Averbeck:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Topic: D.05. Audition

Support: NIH Grant DC014950

DARPA Grant AP00101

Title: Sensory coding properties predict selective attention effects on single units in primary auditory cortex

Authors: Z. P. SCHWARTZ, *S. V. DAVID;
OHRC, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Auditory selective attention is required to discern behaviorally relevant information in crowded acoustic environments. Studies of attention and related behaviors during single-unit recording in animals have revealed diverse behavior-related effects, which have yet to be integrated into a coherent theory of how attention influences neural coding. In order to better understand the diversity of behavioral effects, we asked whether the baseline sensory coding properties of neurons in auditory cortex predict whether they will be modulated by attention. We trained three ferrets to perform an auditory selective attention task. Animals responded to a target tone embedded in one of two simultaneous narrowband noise streams and ignored catch tones in the other. The target stream was switched between blocks, and selective attention was verified by comparing responses to targets versus catch tones. After training, we recorded single-unit

activity in primary auditory cortex (A1) during behavior and during passive presentation of task stimuli (*n*=52). One noise stream was centered at neural best frequency and contralateral to the recording site. Thus, the task alternated between attention directed into the receptive field (*attend RF*) and away from the RF (*attend away*). Identical noise stimuli were played in all behavioral conditions. We compared spiking activity evoked by the noise and tone stimuli in each behavioral condition. During the attend RF condition, the average noise-evoked response decreased. Tone responses did not change. Because only the RF stream evoked neural activity, this change represents a suppression of noise responses at the locus of attention. Distractor responses were suppressed in only about half of recorded units. To test whether a neuron's sensory coding properties predicted modulated by attention, we computed a nonlinear spectro-temporal receptive field (STRF) for each unit. The STRF included a nonlinear input term that accounted for short-term plasticity (STP, depression or facilitation) of sensory inputs. Neurons that showed evidence for STP were less likely to undergo attention-mediate changes. Thus the nonlinear temporal integration properties of A1 neurons predict whether they will undergo changes in tuning during selective attention.

Disclosures: Z.P. Schwartz: None. **S.V. David:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Topic: D.05. Audition

Support: AFOSR FA9550-12-10388

Title: A gradient frequency neural network model of auditory scene analysis

Authors: *J. C. KIM, E. W. LARGE;
Psychological Sci., Univ. of Connecticut, Storrs, CT

Abstract: Acoustic environments typically include multiple sound sources, and it is a central function of the auditory system to segregate individual sounds in an acoustic mixture. This capability, called auditory scene analysis, is important both for understanding how the auditory system works and for developing computational systems capable of detecting and identifying relevant sound events in real-world environments. Computational models of auditory scene analysis commonly employ signal processing techniques to analyze and segregate frequencies in mixture signals. However, a common criticism of the current modeling efforts has been the lack of evidence for biological mechanisms performing such computations. Here we present a

computational model of auditory scene analysis based on the neural processes observed in the auditory system. We model the auditory system as a dynamical system consisting of gradient frequency neural networks (GrFNNs), which are tonotopically organized networks of neural oscillators. We show that self-organizing patterns of synchronized oscillations in GrFNNs provide a biologically plausible account of auditory scene analysis. Oscillators tuned to harmonically related frequencies resonate with each other by mode-locking in integer ratios, so that separate groups of mode-locked oscillators emerge when the GrFNN model is driven by a signal containing multiple harmonic sounds. We show that dynamic pattern formation in the GrFNN model replicates existing empirical data on concurrent sound perception, including the fusion and segregation of concurrent harmonic sounds, the discrimination of concurrent pitches, and the pop out of a mistuned harmonic. This study shows that auditory scene analysis can be achieved by GrFNNs, neural processes known to exist in the auditory system, without introducing external computations based on signal processing techniques.

Disclosures: J.C. Kim: None. **E.W. Large:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Finnish Cultural Foundation

Title: Reward cues direct auditory attention and modulate fMRI activations in monkey auditory cortex

Authors: P. WIKMAN[1], T. RINNE[1], *C. I. PETKOV[2];
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Abstract: Training nonhuman animals on active auditory tasks is notoriously difficult and time consuming, typically requiring hundreds of daily training sessions. Advancing and developing animal models for human auditory cognition critically depends on innovating the approaches for

training nonhuman animals and assessing how auditory-attention related effects influence cortical networks. In this study, we tested whether a novel paradigm based on reward incentive cues, which was originally used to show that monkeys learn a visual categorization task in tens of trials (Minamimoto et al. 2010, *Neuron*), could be adapted to speed up auditory task training and to direct monkeys' auditory attention. First, we trained monkeys to respond to a 400-ms "coo" target sound to receive a juice reward. Then at trial onset, we introduced either high (HiRe) or low (LoRe) reward incentive cues indicating that a correct response would be associated with, respectively, a big and immediate juice reward or a small and delayed reward. We hypothesized that monkeys' performance in the simple auditory target detection task would be systematically better during HiRe than LoRe trials after they learned to discriminate the cues from each other. We compared monkeys' auditory target detection performance when the HiRe and LoRe cues were either two different sounds or visual patterns. Further, in the condition with visual cues, a 2 s tone was played to redirect monkey's attention to the auditory modality after the presentation of the visual cue but before the onset of the "coo" target sound. We found that after only a few training sessions with 100-300 trials, monkey performance showed a significant difference between HiRe and LoRe trials with both auditory and visual cues. However, this difference was systematically bigger, more reliable and faster to achieve when visual incentive cues were used. Thus during fMRI we used the auditory target detection task with visual cues. We hypothesized that the monkeys would attend to the sounds more during HiRe than LoRe trials and, as a result, fMRI activations in auditory cortex would be higher during the HiRe trials. Our results showed that the reward incentive cues significantly biased monkeys' auditory task performance during fMRI. Moreover, activations in auditory cortex were significantly stronger during the HiRe than LoRe trials. Thus, this novel behavioral paradigm successfully revealed activation modulations in the monkey brain associated with focused listening. Remarkably, these results were obtained after less than 15 days of task-specific behavioral training.
*TR and CIP joint senior authors.

Disclosures: **P. Wikman:** None. **T. Rinne:** None. **C.I. Petkov:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 431.06/PP14

Topic: D.05. Audition

Support: UCSD FISP Fellowship

Title: Using deep networks to generate naturalistic stimuli reveals shared higher-level perceptual space among wild-caught starlings

Authors: *M. THIELK[1], T. SHARPEE[2], T. GENTNER[1];
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Abstract: To date, the neural basis of auditory processing is understood primarily in terms of simple sine tones and other artificial signals, and the few cases that have used conspecific natural stimuli rely on serendipitous use of natural variation. This use of clearly impoverished stimuli reflects the failure to understand the relevant acoustic dimensions along which categorical perception varies, which has until now prevented parametric control over the more complex acoustic features composing natural complex stimuli. This lack of control has prevented the application of psychophysical experiments on natural stimuli. Exploiting recent developments in machine learning, we've implemented a novel method to parameterize and explore the auditory stimuli space using a Deep Neural Network. This compressed quantitative representation allows me to generate "morph" motifs between any two arbitrarily chosen song elements (motifs) in a continuous manner which allows me to perform the more complex behavioral manipulations and neurophysiological experiments.
This project explores the neural mechanisms that mediate the recognition of ambiguous auditory objects in European starlings (Sturnus vulgaris) during a categorical perception task. Leveraging this parameterization of the stimuli, we explore how motif categories are encoded within the learned space. We do this by sampling points in this space and constructing categories the birds must learn. We train the subject to associate 4 motifs with right responses and another 4 motifs with left responses using 2AC operant training. We then generate morphs between each of the left motifs and each of the right motifs using my interpolation method giving a total of 16 independent continuous axes to probe along and estimate of their natural perceptual boundary between the left and right category for each morph. We find that the psychometric curves are highly variable within subject across different morph dimensions, yet highly conserved across subjects, which suggests a shared perceptual space between birds. Furthermore, differences in the initial training category parameters result in characteristic and predictable differences in the boundaries. Then, using the pre-trained subjects under anesthesia, we then record from neural populations in different auditory regions to estimate the neurometric functions in response to the morphed stimuli.

Disclosures: **M. Thielk:** None. **T. Sharpee:** None. **T. Gentner:** None.

Poster

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Topic: D.05. Audition

Support: WT102561/Z/13/Z

WT091681MA

Title: Primate BOLD data demonstrating fundamental bases for auditory figure-ground analysis

Authors: *P. DHEERENDRA[1,2], F. BALEZEAU[1], S. KUMAR[1,2], A. BLAMIRE[1], A. THIELE[1], T. D. GRIFFITHS[1,2];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Wellcome Trust Ctr. for Neuroimaging, Inst. of Neurol., Univ. Col. of London, London, United Kingdom

Abstract: A critical aspect of auditory scene analysis is the ability to extract a sound of relevance (figure) from a background of competing sounds (ground) such as when we hear a speaker in a cafe. Previous work on this has used high-level stimuli with stochastic elements like speech in noise or deterministic stimuli like that developed by van Noorden. We have developed a stimulus based on the detection of elements in frequency space in a random background that are repeated over time - a form of sequential grouping of spectral patterns. Functional imaging in humans [1] demonstrates a network that includes auditory cortex and the intraparietal sulcus (IPS).
The stochastic figure ground stimulus we have developed examines fundamental mechanisms for figure ground perception that are equally relevant to the rhesus macaque, in which we can carry out both system level and systematic neuronal specification of the system. We investigated the neural bases of pre-attentive stimulus-driven auditory segregation in rhesus macaques using functional magnetic resonance imaging.
Stimuli were made of 5-15 randomly chosen pure tone components (ground) that change for every chord. This ground segment is overlaid with 10 additional components that are either coherent (figure) or incoherent (control), presented at the middle 2 s of the 6 s long exemplars. EPI images were acquired using a sparse acquisition protocol on 4.7T upright Bruker scanner (TR/TA/TE = 10s/2.01s/21ms) while the animal performed a stimulus irrelevant visual fixation task. 360 volumes (135 each for figure & control) were acquired per session per animal. Analysis was carried out using SPM software (SPM12). Single subject inference was carried out by applying a generalized linear model (GLM).
We observed significant activation in core auditory cortex, anterior and posterior superior temporal sulcus (STS). Our results suggest that analogous to human IPS, macaque posterior STS is involved in mediating pre-attentive auditory segregation identified using an identical stimulus

to that used in previous human study [1].
The data support the use of the macaque as a model for human auditory scene analysis that allows both system-level and neuronal characterisation.
1. Teki, S., et al., Brain bases for auditory stimulus-driven figure-ground segregation. J Neurosci, 2011. 31(1): p. 164-71.

Disclosures: P. Dheerendra: None. **F. Balezeau:** None. **S. Kumar:** None. **A. Blamire:** None. **A. Thiele:** None. **T.D. Griffiths:** None.

Poster

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Support: NARSAD Young Investigator Award

NIMH Grant KMH106744A

Title: Dynamics of cortical activity during behavioral engagement and auditory perception

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Abstract: Changes in brain state can control perceptual abilities by modulating the detection of sensory input in a background of ongoing activity, and the recognition of behaviorally relevant inputs over less relevant or distracting inputs. Neurophysiologially, many aspects of cortical activity and representations can be modulated to affect sensory processing including the structure of neuronal receptive fields, population dynamics, spike rates or spike timing during periods of stimulus presentation, or patterns of spontaneous activity. Depending on the demands of behavioral tasks, there are different modes and levels of engagement that can result in different performance scores. It is not clear how different forms of engagement modulate activity in the auditory cortex to impact perceptual detection and discrimination. Here we examine this question using two different variants of a frequency recognition task while monitoring neural activity in rat auditory cortex.
We recorded 80 single units from the auditory cortex of five rats performing a self-initiated go/no-go auditory task. Self-initiation transformed tuning curves and activity patterns in the auditory cortex. In 55/80 neurons, tone-evoked responses were suppressed while in the rest of the cells they were enhanced. In some cells, self-initiation modulated responses to target and non-

target tones in opposite directions, leading to sharpening of frequency tuning profiles ('self-initiated' $\sigma = 5.6 \pm 1.4$ versus uncued trials $\sigma = 12.5 \pm 3.0$; two-tailed paired t-test, $p<0.02$, n=41 cells). In other cells, self-initiation changed responses to target and non-target tones in the same direction, adjusting thus the gain of the response. Trial self-initiation decreased the rate and the variability of spontaneous activity in 63 of the 80 recorded cells. Optogenetic disruptions of activity patterns in the auditory cortex during the self-initiated trials showed that these changes in spontaneous activity were important for sound perception ('light ON' hit rate, 0.78 ± 0.14 of 'light OFF' trials, $p < 0.05$, two-tailed paired t-test). Thus, behavioral engagement can prepare cortical circuits for sensory processing by sharpening receptive fields and controling the pattern of spontaneous activity.

Disclosures: I. Carcea: None. **M.N. Insanally:** None. **R.C. Froemke:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Topic: D.05. Audition

Support: NIH DC0485

Center for Visual Science

Title: Inactivation of primate dorsolateral prefrontal cortex during auditory working memory.

Authors: *L. M. ROMANSKI[1], B. PLAKKE[1], T. LINCOLN[1], A. POREMBA[2], J. BIGELOW[3]; [1]Dept Neurosci., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; [2]Psychology, Univ. of Iowa, Iowa City, IA; [3]Dept. of Otolaryngology-Head and Neck Surgery, Univ. of California, San Francisco, San Franscisco, CA

Abstract: We have previously shown that inactivation of the ventrolateral prefrontal cortex (VLPFC), including areas 12/47, 45, and ventral 46 results in impaired performance during audiovisual working memory with dynamic vocalization movies as the memoranda. We have also demonstrated that VLPFC inactivation impairs performance when only the auditory stimulus, the vocalization has to be remembered. These experiments demonstrate that VLPFC is crucial in auditory and audiovisual working memory. However, it is also possible that our inactivation may have included portions of the dorsolateral prefrontal cortex (DLPFC). Furthermore, DLPFC may also be involved in these working memory paradigms due to its general role in the process of working memory, and goal directed behavior. We therefore trained

animals to perform an auditory match-to-sample task using 3 categories of complex sounds: macaque vocalizations, other vocal sounds and non-vocal auditory stimuli. Cylinders were aligned over the DLPFC in order to allow for the use of cortical cooling which inactivates the area within the cylinder. For each experimental session the monkeys were brought to the testing room and the auditory match to sample task was started. The subjects performed 100 trials to establish the baseline prior to cooling (WARM trials) then the cooling process was started and the brain was cooled to 20 degrees Celsius in order to reduce synaptic activity and temporarily inactivate the cortex. After 100 trials during cooling (COLD trials) the temperature was returned to normal and the subject was returned to their home cage. Warm and Cold trials were compared in order to determine if cooling of DLPFC has an effect of auditory working memory.

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Poster

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Topic: D.05. Audition

Support: GACR P303/12/1347

GACR P303 16-17823S

Title: The impact of parvalbumin deficiency on auditory function in aging mice

Authors: *J. BURIANOVA[1], R. TURECEK[1], B. SCHWALLER[2], J. SYKA[1];
[1]Dept. of Auditory Neurosci., Inst. of Exptl. Medicine, CAS, Prague, Czech Republic; [2]Anat. Unit, Dept. of Med., Univ. of Fribourg, Fribourg, Switzerland

Abstract: The lack of parvalbumin (PV) leads to behavioral deficits such as impaired social interaction, behavioral flexibility, or altered patterns of locomotor activity. In comparison with wild type mice (WT - C57Bl/6J), parvalbumin-deficient (PV-/-) mice exhibit alterations in acoustic startle response (ASR) and prepulse inhibition (PPI). It remains to be elucidated, however, whether these changes persist throughout the life span of the mouse or whether they manifest any age dependent specificity. The present study was aimed at exploring the effects of PV deficiency on ASR and PPI using juvenile (1 month-old), young adult (5 month-old) or aged (16 month-old) mice. The ASR in response to a broad-band noise impulse or a tone pip (4, 8, 16 kHz) and the PPI of ASR elicited by a broad-band noise impulse or 4, 8, or 16 kHz tones over a

range of 20-80 dB SPL were recorded. Hearing function in the mice was evaluated by recording the auditory brainstem responses (ABRs). In juveniles and young adults, no difference was found between WT and PV-/- mice in their ABRs. Unexpectedly, aged PV-/- mice showed ABR thresholds similar to young adults while aged WT mice had their hearing thresholds elevated across all frequencies tested. The ASR and PPI of the juveniles were comparable in both groups. In young adults, WT mice exhibited increased ASR to broad-band noise and 8 kHz tones, while PV-/- mice responded more to 16 kHz tones. Furthermore, PPI of ASR was more efficient in WT than PV-/- young adult mice across all used prepulse frequencies and intensities. In aged animals, the pattern of ASR and PPI results was found to be reversed. The PV (-/-) mice exhibited more efficient PPI (particularly when using a broad-band noise impulse) and significantly pronounced ASR to 120 dB pips, which might suggest the appearance of hyperacusis. Taken together, the data supports the importance of parvalbumin for auditory functions and suggests the novel role of this protein in processes associated with aging of the mammalian auditory system. The underlying mechanisms deserve to be explored in more detail.

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Poster

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Topic: D.05. Audition

Support: T32 NS041218-14

Title: DREADD-mediated silencing of projections from basolateral amygdala to nucleus accumbens disrupts pre-pulse inhibition in rats.

Authors: ***B. L. AGUILAR**[1,2,3], E. WICKER[3,1], L. MALKOVA[3,1,2], P. A. FORCELLI[2,3,1]; [2]Interdisciplinary Program in Neurosci., [3]Dept. of Pharmacol. & Physiol., [1]Georgetown Univ., Washington, DC

Abstract: Sensorimotor gating is a fundamental process through which the central nervous system filters motor responses to redundant or irrelevant stimuli; this process can be assessed operationally through measurement of prepulse inhibition (PPI) of the acoustic startle response (ASR). PPI is a well-conserved effect across species in which the magnitude of ASR is attenuated by presentation of a low-intensity prepulse prior to the startle-inducing stimulus. PPI deficits have been reported in a number of neuropsychiatric disorders. Investigations of circuitry underlying PPI have relied primarily on lesion and pharmacological inactivation studies, both of

which lack the ability to target circuitry in a projection-specific manner. The use of chemogenetic technology allows pathway specific targeting through the use of designer receptors exclusively activated by designer drugs (DREADDs). These receptors allow for the selective inactivation of DREADD-expressing neurons by otherwise inert compounds (clozapine-n-oxide, CNO, hM4D agonist). Our lab has previously reported (Forcelli et al., 2012) that inhibition of basolateral amygdala (BLA) disrupts PPI, and that this effect is mediated by the ventral pallidum (VP). However, BLA does not project directly to VP, but rather through a relay in the nucleus accumbens (NAcc). Here, we sought to test the hypothesis that inhibition of projections from to NAcc would be sufficient to impair PPI, providing functional evidence for the proposed circuit. 12 Long Evans rats were injected unilaterally into BLA with AAV-hSyn-hM4D(Gi)-mCherry DREADD construct paired with an ipsilaterally placed cannula targeted to the NAcc. Using an SR-LAB rodent startle response system, we determined that silencing of BLA projections terminating in the NAcc disrupted sensorimotor gating as measured by a reduction in PPI. Rats were treated with either systemic CNO (10mg/kg) or with intra-NAcc injection of CNO (1nmol). The former treatment was intended to silence all BLA projections, while the latter targeted BLA terminals in NAcc. Intracerebral microinfusions of 1mM CNO resulted in a significant decrease in PPI at prepulse intensities of 3 and 6dB above background ($p<0.05$). Additionally, systemic injections at a dose of 10mg/kg CNO yielded a significant decrease in PPI at a prepulse of 3dB above background ($p<0.05$). These results demonstrate that DREADD-mediated silencing of the direct BLA to NAcc pathway results in a disruption of sensorimotor gating function as quantified by a loss of prepulse inhibition to the acoustic startle, and suggest that projections from BLA to NAcc are sufficient to account for the PPI-disruptive effects of BLA silencing.

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Poster

431. Auditory Processing and Perception in Non-Humans

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Title: Attenuated responses to self-generated sounds in auditory cortex

Authors: B. RUMMELL, J. KLEE, *T. SIGURDSSON;
Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract: Many of the sounds that we perceive are caused by our own actions, for example during speech or movement, and must be distinguished from sounds caused by external events. Studies using macroscopic measurements of brain activity in human subjects have consistently shown that responses to self-generated sounds are attenuated in amplitude. However, the underlying manifestation of this phenomenon at the cellular level is not well understood. In order to address this, we recorded the activity of neurons in the auditory cortex of mice in response to sounds that were generated by their own behavior. We found that the responses of auditory cortical neurons to these self-generated sounds were consistently attenuated, in comparison to the same sounds generated independently of the animals' behavior. This effect was observed, with differences in magnitude, in upper and lower layers of auditory cortex as well as in putative pyramidal neurons and interneurons. Downstream of the auditory cortex, we found that responses of hippocampal neurons to self-generated sounds were almost entirely suppressed. Responses to self-generated optogenetic stimulation of thalamocortical terminals were also attenuated, suggesting a cortical contribution to this effect. Further analyses revealed that the attenuation of self-generated sounds was not simply due to the non-specific effects of movement or behavioral state on auditory responsiveness. However, the strength of attenuation depended on the degree to which self-generated sounds were expected to occur, in a cell-type specific manner. Taken together, these results reveal the cellular basis underlying attenuated responses to self-generated sounds and suggest that predictive mechanisms contribute to this effect.

Disclosures: **B. Rummell:** None. **J. Klee:** None. **T. Sigurdsson:** None.

Poster

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Title: Behavioral uncertainty in tone-in-noise and speech-in-noise detection tasks reflected in neuronal responses in ferret auditory and frontal cortices

Authors: ***J. B. FRITZ**[1], C. BIMBARD[2], D. D. DUQUE[1], D. DELGUEDA[3], S. V. DAVID[4], S. A. SHAMMA[3];
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Abstract: The detection of faint sounds in the presence of background noise is a ubiquitous acoustic challenge in active hearing for humans and animals. In a previous study (Atiani et al., 2009) ferrets (*Mustela putorius*) were trained on a conditioned avoidance go-nogo task to detect a tone embedded in noise. The level of difficulty of the tone-in-noise (TIN) task was parametrically varied by changing the signal-to-noise ratio (SNR) of the pure tone relative to background noise. Ferrets took longer to recognize the presence of the tone as SNR decreased, reflecting rising difficulty of extracting signals from increasingly noisy surroundings and uncertainty in low SNR as to whether the tone was present. We also observed rapid task-related plasticity in primary auditory cortex (A1) that may enable better TIN detection. Other work (Mesgarani et al., 2014) has shown a noise-robust representation of speech, suggesting that extraction of signal from noise can be studied in the ferret for more complex acoustic stimuli such as speech (Doncos et al., 2016). The objective of the present study was to explore neural responses in higher auditory cortical areas beyond A1 during task performance and elucidate contributions of different areas to neuronal recognition of the target tone (TIN task) or speech token. We recorded neuronal activity extracellularly, with high impedance electrodes, from A1, higher order auditory cortex (the ventral posterior area (VP)), and from dorsolateral frontal cortex (dlFC) in four female adult ferrets trained on the TIN and the speech-in-noise task. We recorded over 110 single units in dlFC under quiescent conditions and in two behavioral task conditions of pure tone detection, and TIN, with variable SNR. Frontal neurons during TIN behavior showed: (1) similar selective responses to the tonal target, even when embedded in noise, (2) greater latency of target response in the TIN task compared to target latency in pure tone detection reflecting greater processing time to detect the TIN, (3) diminished amplitude of target responses in the TIN task compared to target responses in pure tone detection, reflecting a greater degree of uncertainty about the presence of target tonal stimuli. Similar responses in the TIN task were also observed in 40 cells recorded in VP. Results were also obtained from A1, VP and dlFC in one female ferret trained on the speech-in-noise task with variable SNR. The intriguing parallel between task performance and neural responses in frontal cortex and VP and the adaptive A1 receptive field responses suggests that all areas contribute to a broader auditory network for extraction of relevant signals from noise.

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Poster

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Title: Higher age-related decline in behavior discrimination of amplitude modulation frequencies compared to auditory evoked potentials

Authors: *J. LAI[1], E. L. BARTLETT[2];
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Abstract: Age-related hearing loss, or presbyacusis, causes a reduction in speech understanding which could eventually result in self-isolation, loneliness and depression. Speech consists of complex and rapid amplitude modulation (AM) and frequency modulation, so the ability to discriminate between modulation frequencies is important for discrimination of speech and other complex sounds. It is known that age-related differences in temporal processing assessed physiologically by envelope following responses (EFRs) of the auditory midbrain and brainstem are significant at more rapid AM frequencies. A typical assumption is that a decline in EFR responses will necessarily result in corresponding perceptual decline. To test this assumption, we investigated behavior discrimination abilities of AM frequencies in young and aged Fischer-344 rats. Comparison of physiological and behavior results should aid in reflecting the relationship of AM processing and AM perception. For AM processing, we measured EFRs of temporal modulation transfer function (tMTF) using sinusoidal AM (SAM) 8 kHz tone at AM frequencies of 16-2048 Hz in 0.5-octave step. For behavior AM discrimination, we used a modified version of prepulse inhibition (PPI) of acoustic startle reflex (ASR). In a typical trial of AM discrimination task, pulses of SAM tone at 128 Hz AM frequency were presented sequentially and a prepulse of SAM tone at the same or different SAM frequency (e.g. 32, 256 Hz, etc.) was presented right before a sudden brief loud noise that induced ASR. Larger PPI intrinsically indicates a higher sensitivity in discriminating SAM frequency of the prepulse from 128 Hz. Comparable behavior PPI results were observed in the young and the aged when the AM discrimination task was performed at 100 % AM depth even though age-related differences in EFR amplitudes of tMTF at 100 % depth were large. At 50 % AM depth, age-related decline of EFR amplitudes was small but aged animals' AM discrimination performance was highly compromised. In contrast, young animals were able to discriminate AM frequencies at 50 % depth though their EFR amplitudes were slightly higher than the aged. Even at 25 % depth, young animals were still able to discriminate AM frequencies that are about 3 octaves away from 128 Hz. Overall, the results reveal a complex relationship between EFRs and behavioral age-related differences, suggesting that EFR amplitude or changes in EFR amplitude alone are not enough to predict behavioral performance.

Disclosures: J. Lai: None. **E.L. Bartlett:** None.

Poster

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Title: Parallel processing by cortical inhibition in auditory cortex enables flexible behavior and learning

Authors: *K. KUCHIBHOTLA[1], J. V. GILL[1], G. W. LINDSAY[2], E. PAPADOYANNIS[1], T. A. H. STEN[1], R. E. FIELD[1], K. D. MILLER[2], R. C. FROEMKE[3];
[1]Skirball Inst., New York Univ. Sch. of Med., New York, NY; [2]Columbia Unviersity, New York, NY; [3]NYU Sch. of Med., New York, NY

Abstract: Neocortical inhibitory neurons are remarkably diverse in terms of lineage and connectivity, but little is known about how this diversity supports perception and behavior. Here we show that during a stimulus recognition task, excitatory neurons in auditory cortex exhibit a continuum of responses about a mean suppression, with high variance including some response facilitation. With whole-cell recording, two-photon calcium imaging and optogenetics during behavior, we found that synaptic inhibition gated these changes, with PV, SOM+ and VIP+ interneurons dynamically balancing inhibition and disinhibition within the network. Cholinergic axons increased activity during behavior, directly depolarized all interneuron subtypes, and were necessary for behavioral performance. Stimulating the cholinergic system elicited fictive behavioral responses even out-of-context. A network model captured the complex neural dynamics across neuronal subtypes only when cholinergic activity coincidently depolarized all interneurons. Differential responses of cortical interneurons to neuromodulation may thus enable circuits to have distinctive responses during behavior. We next explored excitatory neural dynamics during the acquisition of this flexible task. Neural correlates to stimulus recognition and behavioral context emerged early, well before the animal could behaviorally report task contingencies correctly in the active context. This suggests that sensory learning may be dissociable from complete task acquisition.

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Poster

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Sumitomo Foundation

Title: Acoustic environmental enrichment prolonged natural lifespan of mice

Authors: *Y. YAMASHITA[1], N. KAWAI[2], O. UENO[1], T. OOHASHI[2], M. HONDA[1];
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Abstract: Environmental enrichment (EE) has been reported to have positive effects on experimental animals including prevention of frailty signs, prolongation of lifespan, and accelerations of developments and functions of the brain. A standard experimental setting of EE consists of the interaction of complex factors including larger cages with variety of objects enhancing animal's physical activity and larger groups of animals with the opportunity for more social interaction. In such complex factors, diversity of sensory stimuli may be one of the essential factors of enriched environment. For example, recent studies have demonstrated that enrichment of acoustic environment promotes plasticity and recovery of the auditory cortex. In the current study, we focused on the effects of enrichment of acoustic environment and investigated whether acoustic enrichment affects lifespan and voluntary movements in mice. Ninety-six 8-week old C57Bl/6J mice were assigned to two groups with different acoustic environments throughout the experiment: (1) acoustically enriched environment with sounds of tropical rain forest (AEE group, n=64, 32-male and 32-female), and (2) standard environment without any exposure to stimulation (control: CNT group, n=32, 16-male and 16-female). In AEE condition, tropical rain forest sounds were presented through two speakers fixed at the top of the cage. Other properties of environment including cage size (a standard plastic cage) were standard rearing condition of experimental animals. Body weights were monitored once every week, and voluntary movements were continuously monitored with a computerized system throughout lifespan.
The mice of AEE group lived significantly longer (nearly 12%) than those of CNT group. In addition, the voluntary movements of AEE group significantly increased compared to those of CNT group. However, no correlation between the lifespan and voluntary movements was observed. There was no significant difference in body weights between AEE group and CNT

group.
The results showed that additional acoustic stimuli with standard rearing condition prolonged natural lifespan of the mice. One may argue that prolonged lifespan might result from increased voluntary movements. However, there was no significant correlation between the voluntary movements and lifespan, suggesting that the increase of voluntary movements was not a primary cause of prolongation of lifespan. These observations suggest that acoustic sensory enrichment may be one of the essential elements of EE. This work could contribute to providing clues regarding underlying mechanisms of the positive effects of EE.

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Poster

431. Auditory Processing and Perception in Non-Humans

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 431.17/QQ9

Topic: D.05. Audition

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS), Japan Agency for Medical Research and development (AMED)

Brain Science Project of the Center for Novel Science Initiatives (CNSI), National Institutes of Natural Sciences (NINS) (BS271009)

Title: Shared mechanisms of mismatch activity in common marmosets and macaque monkeys

Authors: ***M. KOMATSU**, N. FUJII;
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Abstract: Mismatch negativity (MMN) is a component of event-related potentials evoked by violations of the regularity in sensory stimulus-sequences in human. MMN has been receiving attentions as a clinical and translatable biomarker of psychiatric disorders such as schizophrenia. Recently many studies are focusing on MMN of several species to develop the animal models of these psychiatric disorders. In this study, we investigated shared mechanisms of MMN in two species of non-human primates, common marmosets and macaque monkeys. We recorded the electrocorticograms (ECoGs) from four common marmosets and three macaque monkeys with implanted electrodes covering a wide range of a hemisphere. The 28-64 channel ECoG arrays were epidurally implanted on the hemispheres of each marmoset (two on the left and two on the right hemispheres) and the 128 channel arrays were subdurally implanted on the brains of the

macaque monkeys (one on the left and two on the right). ECoG recordings were conducted in passive listening condition with a roving oddball paradigm. Repetitive tone-sequences with 20 types of frequency (250-6727 Hz with an interval of 1/4 octaves) are randomly presented. We considered the last tones of sequences as standard, and the first tones of sequences as deviants. First, we calculated ERPs for standards and deviant stimulus, respectively. Then, difference wave is obtained by subtracting the deviant stimulus ERP from the standard stimulus ERP. We observed significant negative components of the difference wave lasting 40-150 ms after the onset of the stimuli, around the lateral sulcus of both the species. This result suggested that shared mechanisms of mismatch activity exist in information processing around the lateral sulcus of both the species. Furthermore, we investigated the functional connectivity between electrodes of a marmoset. We calculated direct Directed Transfer Function (dDTF) during 0-100 ms after the onsets of the standard and deviant stimuli, respectively. At the high frequency band (56~ Hz), intra connections appeared in the temporal area during both the period, while prominent connections in the frontal area appeared for the deviant stimuli period, but not for the standard stimuli period. These results suggested the high-gamma activity reflected different roles of temporal and frontal generator.

Disclosures: **M. Komatsu:** None. **N. Fujii:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Topic: D.05. Audition

Support: NIH Grant DC012124

NIH Grant DC014950

Title: Task-related plasticity in the inferior colliculus of the marmoset monkey

Authors: ***S. J. SLEE**, S. V. DAVID;
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Abstract: Recent evidence suggests that neurons in the inferior colliculus (IC) undergo receptive field changes during auditory behavior in carnivores (Slee and David, 2015. J Neurosci.). In this study, we tested for similar effects in the marmoset monkey, a primate species that uses vocalizations to communicate. Two marmosets were trained to detect a pure tone target embedded in a background of random spectral shape (RSS) distractor stimuli. The level of the

target was manipulated to vary task difficulty. Both marmosets accurately performed this task over a 5-octave range of target frequencies (0.625-20 kHz). As the signal to noise ratio of the target was decreased, we found a significant decrease in hit rate and an increase in false alarm rate. We measured the effects of task engagement by recording from single neurons in the IC. Neural responses to both targets and RSS distractors were compared between conditions when the marmoset performed the detection task or listened passively. The target frequency was presented near the best frequency (BF) of the neuron under study. Responses to the distractors were suppressed in about half of the neurons during task engagement relative to passive listening. The median global gain change (-14%) in these neurons was comparable to our previous study in the ferret (median=-20%). To measure local tuning changes, responses to the RSS distractor stimuli were also used to fit linear and nonlinear spectral weighting models. Spectral weights were tuned around BF for most neurons in the central nucleus of the IC. In about 1/3 of these neurons we found a significant decrease in the spectral weight at BF (target frequency) during task engagement. The median weight change (-25%) was also similar to previous measurements in the ferret (-32%). Finally, we computed the discrimination index (*d'*) between the distributions of neural responses to the target and distractor stimuli in both behavioral conditions. We found that while most IC neurons can discriminate between the task stimuli (*d'*>1), discrimination *does not* improve during task engagement. These results support a model with task-related plasticity in the IC as a prerequisite for the improved neural discrimination that has been reported in auditory cortex.

Disclosures: S.J. Slee: None. **S.V. David:** None.

Poster

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Topic: D.05. Audition

Support: NIDCD-NIH

Title: Contribution of population activity in the auditory cortex to the cocktail-party problem

Authors: *K. L. CHRISTISON-LAGAY[1], S. BENNUR[2], Y. COHEN[2];
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Abstract: One of the fundamental functions of the auditory system is to transform acoustic stimuli into discrete perceptual representations (sounds). However, this task is often complicated

by our listening conditions: rarely do we listen to sounds against silence. Instead, our auditory system often must extract and segregate a “target” stimulus from a noisy background (e.g., isolating your friend’s speech from the rest of the sounds in a restaurant). This ability is frequently referred to as figure-ground segregation or, more informally, as the cocktail-party problem. The neural underpinnings of this ability, specifically those in the auditory cortex, have not yet been fully elucidated. To address this issue, we recorded from the auditory cortex while rhesus monkeys simultaneously reported whether they heard a target stimulus that was embedded in a noisy background. The target stimulus was a tone burst, whose frequency was set to the best frequency of the recording site, and the noisy background was comodulated broadband noise. On a trial-by- trial basis, we varied the “target-in-noise” ratio (TNR) between 0 and 25 dB. On approximately of 50% of the trials, we presented the noise alone; these trials served as catch trials. Although most auditory-cortex neurons were driven by the auditory stimuli, only a small fraction of neurons was significantly modulated as a function of TNR levels. Further, we could not identify a significant population of neurons that was modulated by the monkeys’ choices (detection versus no detection of the target stimulus). Because of these two sets of findings, it seemed that very few individual neurons contributed sensory evidence to the task’s perceptual decision. However, the heterogeneity of neural responsivity suggested that population activity might provide more stimulus- and/or task-related information. To test this hypothesis, we constructed a support vector machine and tested how well neural activity in response to different stimulus and/or task attributes could be discriminated using a linear-decision- boundary. With populations as small as ~100 neurons, this classifier could reliable decode TNR level, discriminate between the target and noise stimulus, and discriminate between variability that was modulated by the monkeys’ choices. Overall, these findings are consistent with the hypothesis that auditory cortex does contribute directly to the cocktail-party problem. However, this contribution is not seen in individual neurons but the overall activity of the population.

Disclosures: **K.L. Christison-Lagay:** None. **S. Bennur:** None. **Y. Cohen:** None.

Poster

431. Auditory Processing and Perception in Non-Humans

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Topic: D.05. Audition

Support: Office for Research and Innovation at the University of Oregon

Title: The role of auditory thalamo-striatal and cortico-striatal neurons in amplitude modulation frequency discrimination

Authors: *N. D. PONVERT, S. JARAMILLO;
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Abstract: Multiple parallel neural pathways link information about sounds with behavioral responses. The striatum, a brain structure involved in movement and reward-related learning, receives neuronal projections from both the auditory thalamus and the auditory cortex. It is unclear whether sound information that reaches the striatum through these two parallel pathways is redundant or complementary. To test whether both pathways are required for auditory discrimination of amplitude modulated sounds, we first performed transient pharmacological inactivations of auditory cortex in mice trained to perform a two-alternative choice auditory task. We then used optogenetic techniques to tag neurons in the thalamo-striatal and cortico-striatal pathways, allowing us to identify them during extracellular recording. We characterized the responses of identified neurons in each pathway to pure tones and to amplitude-modulated noise stimuli. Consistent with previous studies, we found that inactivation of auditory cortex largely impaired the ability to discriminate between different amplitude modulation frequencies. We also found that neurons in the thalamo-striatal and cortico-striatal pathways have largely overlapping tuning for sound frequency, but display different coding strategies for amplitude modulated sounds. These results suggest that the thalamo-striatal pathway carries sufficient information to support discriminations of sound frequency, but that fundamental differences exist between the cortico-striatal and thalamo-striatal pathways with respect to their contribution to discrimination of temporal modulations in amplitude.

Disclosures: N.D. Ponvert: None. **S. Jaramillo:** None.

Poster

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Topic: D.05. Audition

Support: ERC ADAM E-138

Title: Behavior-dependent gating and extraction of task-relevant auditory signals in ferret frontal cortex

Authors: *J. LAWLOR BLONDEL[1,2], B. ENGLITZ[3], A. MEYER[4], U. GÓRSKA[3], S. SHAMMA[1,2], Y. BOUBENEC[1,2];
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Nijmegen, Nijmegen, Netherlands; [4]Gatsby Computat. Neurosci. Unit, Sainsbury Wellcome Ctr., London, United Kingdom

Abstract: The frontal cortex has been previously associated with enhancement of relevant information for goal-directed behavior. Neuronal responses in the frontal cortex (FC) of the behaving ferret have been shown to be behaviorally gated and highly selective for target stimuli during auditory and visual discrimination tasks (Fritz et al., 2010). This suggests a selection in the flow of sensory information by frontal cortex in tasks in which ferrets rapidly categorize reference and targets. However, in a more natural and cluttered environment, accumulating relevant evidence to produce an adequate behavior is critical. Here we investigate how FC can extract targets embedded in continuous sound stream, and how FC is involved in a complex task demanding accumulation of sensory evidence. For this purpose we trained ferrets on a change detection paradigm where animals have to constantly monitor a stochastic and continuous acoustic stream to detect subtle statistical changes. In an attempt to characterize the extraction of relevant sensory information performed between sensory cortices and frontal areas, we gathered electrophysiological data in the primary auditory (A1) cortex and the dorso-lateral FC (dlFC) of the behaving ferret. A1 neurons exhibited strong onset responses and reduced change-related discharges, whereas dlFC neurons presented an enhanced response to change-related events during behavior, possibly being the signature of accumulation of sensory evidence. These area-specific responses to sound are consistent with EEG recordings done in humans performing the same task. All together this suggest a behavior-dependent sensory 'gating' mechanism leading to decision making.

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Poster

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Topic: D.05. Audition

Support: NIDCD-NIH

Title: Contribution of correlated neural activity in the auditory cortex to the cocktail-party problem

Authors: F. RODRIGUEZ-CAMPOS[1], T. BANNO[1], *Y. E. COHEN[2], S. BENNUR[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept. Otorhinolaryngology-Head Neck Surgery, Univ. of Pennsylvania Dept. of Otorhinolaryngology, Philadelphia, PA

Abstract: One of the fundamental functions of the auditory system is to transform acoustic stimuli into discrete perceptual representations (sounds). However, often, because we listen in noisy environments, our auditory system needs to extract and segregate a "target" stimulus (e.g., your friend's speech) from a noisy background (e.g., the sounds that are formed by other talkers in a restaurant). This ability is often referred to as figure-ground segregation or, more informally, as the cocktail-party problem. However, it is unknown whether and if coordinated correlated auditory contributes to the computations underlying the cochtail-party. To address this issue, we recorded from the auditory cortex while rhesus monkeys simultaneously reported whether or not they heard a target stimulus that was embedded in a noisy background. The target stimulus was a tone burst, whose frequency was set to the best frequency of the recording site, and the noisy background was comodulated broadband noise. On a trial-by-trial basis, we varied the "target-in-noise" ratio (TNR) between 0 and 25 dB. On approximately 50% of the trials, we presented the noise alone; these trials served as catch trials. We recorded with Plexon u-probes and were able to isolate several neurons in each recording session. In general, we did not identify any changes in correlated activity with changes in TNR level. However, changes in correlated activity did emerge as a function of the monkeys' performance. In particular, we found that signal correlation was modulated as a function of task performance (hits versus misses). Similarly, noise correlation was modulated as function of whether the monkeys' reported a hit versus a miss. Finally, we found that the Fano factor (an index of spiking variability) was smaller on hit trials than on miss trials. These findings suggest changes in coordinated population activity may limit a listener's performance on tasks like foreground-background segregation. They also suggest that changes in Fano factor may be an underlying mechanism.

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Poster

432. Human Visual Cortex

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NIH 1U54MH091657

Indiana University College of Arts and Sciences

Title: Decoding the white matter geometrical structure by encoding connectomes in multidimensional spaces

Authors: *F. PESTILLI[1], C. F. CAIAFA[2];
[1]Psychology, Neuroscience, Cognitive Sci. and Network Sci., Indiana Univ., Bloomington, IN;
[2]Psychological and Brain Sci., Indiana Univ. / CONICET, Bloomington, IN

Abstract: A growing body of scientific evidence suggests the inner workings of the brain cannot be simply understood at the level of single neurons or isolated brain areas; rather, modern efforts must focus on brain networks and circuit behavior. The full network of brain connections, commonly referred to as the connectome, is comprised of both grey matter regions representing neural units of information processing, and white-matter tracts serving as structural communication pathways. To date, large-scale human brain networks can be mapped in-vivo by using diffusion- weighted magnetic resonance imaging and fiber tracking methods (Bullmore and Sporns 2009). Standard methods for mapping connectomes operate on data based on the naturally occurring geometry of the brain white matter. This geometry is complex and difficult to handle computationally. As a result a majority of algorithms for mapping connectomes rely on sets of ad-hoc rules and heuristics that identify white matter pathways one fascicle at the time. This is a computationally suboptimal process prone to failure because no rule is expected to be optimal in all brain locations or across multiple brains (Takemura et al., 2016). Computational limitations restrict our ability to routinely measure the accuracy of connectome models (Pestilli et al., 2014).We introduce an approach that uses multidimensional arrays to encode anatomical properties of connectomes in a computationally-efficient way. The method allows performing neuroanatomical operations on the geometric organization of the brain's white matter. We analyzed 1,040 brain connectomes generated using multiple tractography algorithms on publicly available datasets (Human Connectome Project, purl.stanford.edu/ng782rw8378). Results show that the framework: (1) Can be used to establish approaches for precision connectomics by allowing building highly reliable within-brain and highly discriminable between-brain connectomes; (2) Allow efficient estimation of distributions of crossing angles between white matter fascicles (Van Wedeen et al., 2012; Catani et al., 2012). In sum, we show a connectome encoding framework with important computational advantages for decoding fundamental features of the human brain.

Disclosures: F. Pestilli: None. **C.F. Caiafa:** None.

Poster

432. Human Visual Cortex

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Title: Towards a standard cortical observer model in human V1-V3

Authors: C. OLSSON, 10003[1], N. C. BENSON[2], *J. WINAWER[2];
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Abstract: A goal of visual neuroscience is to be able to predict neural responses to arbitrary images. There has been considerable success modeling signals in human visual field maps at the level of fMRI. Population receptive field ('pRF') models can predict responses in a voxel to a wide variety of images (e.g., Kay et al, 2013, 10.1371/journal.pcbi.1003079). These models are solved independently for each voxel by learning the pRF parameters that best fit fMRI measurements. Retinotopic templates are a complementary type of model. They can predict the preferred position in the visual field of all voxels in V1-V3 from anatomy alone, but cannot predict responses to arbitrary images (Benson et al, 2014, 10.1371/journal.pcbi.1003538). Here we integrated a pRF model with an anatomical template to create a first generation 'Standard Cortical Observer Model of Human V1-V3'. The pRF component is based on the two-stage cascade model of Kay et al (2013), and has been extended to handle multiple spatial frequency bands. The pRF parameters summarize the voxel's sensitivity to a variety of image features, including spatial location, spatial frequency, and second order contrast. The V1-V3 template component is an extension of Benson et al (2014). The complete model takes visual images and an anatomical MRI as input. It outputs the predicted BOLD response of each voxel in V1-V3 for each image. The model accomplishes this by: (1) identifying voxels in V1-V3 and deriving their retinotopic coordinates from the anatomical MRI, (2) inferring additional pRF parameters from the retinotopic coordinates, and (3) applying the pRF model at each voxel to the images. The mapping between retinotopic coordinates and other pRF parameters was learned by identifying systematic variation of pRF parameters across voxels in training data. Once learned, the mapping is applied to new subjects and new images with no further fMRI measurements. The model and training data are publicly available, with code on GitHub and a working version packaged in a Docker. The code is structured to facilitate model comparison, such that other researchers can easily extend or modify the model, or apply it to their own data. In preliminary testing, we predicted responses in all voxels in one subject's V1, V2, and V3 to a set of 57 images. The inputs to the model were the images and the subject's unlabeled anatomical MRI. The accuracy of the predicted responses averaged across V1 was 61% (R^2). The model has the combined

advantages of a pRF model and a template, in that it predicts responses to arbitrary images, can be computed from anatomy alone, and takes advantage of the fact that model parameters are distributed systematically on the cortex.

Disclosures: **C. Olsson:** None. **N.C. Benson:** None. **J. Winawer:** None.

Poster

432. Human Visual Cortex

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Support: McDonnell Center for Systems Neuroscience and Arts & Sciences at Washington University

Title: A fully computable model of bottom-up and top-down processing in high-level visual cortex

Authors: *K. N. KAY[1], J. D. YEATMAN[2];
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Abstract: Specific regions of ventral temporal cortex (VTC) appear to be specialized for the representation of certain visual categories: for example, the visual word form area (VWFA) for words and the fusiform face area (FFA) for faces. However, a computational understanding of how these regions process visual inputs is lacking. Here we develop a fully computable model of responses in VWFA and FFA. We measured BOLD responses in these regions to a wide range of carefully controlled grayscale images while subjects performed different tasks (fixation task: judge color of a small central dot; categorization task: report perceived stimulus category; one-back task: detect image repetitions). Using cross- validation to control for overfitting, we developed a model that accurately accounts for the observed data. The first component of the model is a two-stage cascade of visual processing in which the bottom-up response in VTC (fixation task) is computed as the degree to which low-level stimulus properties match a category template. This reveals how high-level representations are constructed from simple stimulus properties. The second component of the model addresses top-down enhancement of VTC responses produced by performance of a task on the stimulus (categorization and one-back tasks). We show that the enhancement is stimulus-specific and can be modeled as a scaling of the bottom-up representation by the intraparietal sulcus (IPS). The third and final component of the model shows that the IPS response to a given stimulus reflects perceptual decision-making and can be quantitatively predicted using a drift diffusion model. Thus, the top-down scaling induced

by the IPS is directly related to the behavioral goals of the subject. In sum, these results provide a unifying account of neural processing in VTC in the form of a model that addresses both bottom-up and top-down effects and quantitatively predicts VTC responses.

Disclosures: K.N. Kay: None. **J.D. Yeatman:** None.

Poster

432. Human Visual Cortex

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Title: Preserved information in multivoxel patterns despite significant decrease in mean signals following surgical removal of human inferior occipital cortex

Authors: *K. S. WEINER[1], J. JONAS[2], L. MAILLARD[2], G. HOSSU[3], S. COLNAT-COULBOIS[2], K. GRILL-SPECTOR[1], B. ROSSION[4];
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Abstract: The stable and plastic features of cortical repair following damage to the human brain are a major topic in systems neurobiology. However, there is little understanding regarding how partial removal of a brain region affects the functionality of the remaining region due to the rarity of acquiring brain measurements pre-damage in humans. Here, we had the unique opportunity to fill this gap in knowledge with pre- and post-operative functional magnetic resonance imaging (fMRI) in a patient (SP) requiring focal resection of the inferior occipital cortex in the right hemisphere. Despite the focality of the surgery, four brain regions were partially resected: a majority of hV4 (66.2%) and less than a quarter of VO-1 (16.1%), pFus-faces/FFA-1 (23.3%), and LOS-limbs/EBA-1 (21.7%). We asked: (1) How does the resection affect mean signal and multivoxel patterns (MVP) in the tissue adjacent to the resection? (2) Do these effects differ in regions that are category-selective (e.g. pFus-faces/FFA-1 and LOS-limbs/EBA-1) compared to

those that are not (e.g. hV4 and VO-1). In four fMRI sessions (two before surgery and two after surgery), SP participated in two experimental runs during which she viewed images from four categories (faces, limbs, places, objects), as well as phase-scrambled images. We measured and compared both mean responses and MVPs in SP pre- and post-operatively, which revealed two main findings. First, partial resection detrimentally affects global processing: the mean signal within each region significantly decreases post-resection irrespective of both the proportion of brain tissue removed, as well as the functional selectivity of the region. Second, distributed representations to object categories were preserved despite a decrement in the mean signal: representational similarity among MVPs is preserved and comparable to pre-resection representations if less than a quarter of the region was removed but not if more than 2/3 of the region was removed (as in hV4). These rare data indicate that the functional assessment of regions surviving cortical resection varies across spatial scales and is sensitive to the amount of the functional region that has been removed. Together, these results caution conclusions of diminished function of a brain region based solely on global reductions in the mean fMRI signal following cortical resection or damage.

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Poster

432. Human Visual Cortex

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Support: NWO Research talent 406-12-141

Title: Population receptive field attraction by spatial attention varies across cortical depth in human V1

Authors: ***B. P. KLEIN**[1], A. FRACASSO[2,3], S. O. DUMOULIN[1,2];
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Abstract: Introduction:
Attention is the mechanism through which we select relevant information from our visual environment. Recently, we have demonstrated that voluntary spatial attention attracts population receptive fields (pRFs) towards its focus in humans across the visual hierarchy, including primary visual cortex (V1) (Klein et al, 2014). Data from non-human primate histology shows

that in V1, visual processing is functionally segregated across different cortical layers at the level of the first synapse. Central layers predominantly receive ascending feedforward inputs, whereas descending feedback inputs mainly terminate in deep and superficial layers. Because of the separation between feedback and feedforward inputs in V1, we expect that attention affects pRF positions in human V1 differently between cortical layers.
Methods:
We acquired sub-millimeter (0.7 mm isotropic, TR = 4s) ultra-high field (7T) functional MRI data while participants viewed an expanding, contrast-defined ring, centered on fixation (±5.5 degrees radius). Concurrently, participants performed an endogenous attention demanding contrast discrimination task on one of two locations, either left or right outside the stimulus range. We estimate pRF positions from the BOLD-fMRI time-series elicited by the expanding ring stimulus for both task locations separately and compare these estimates between the two conditions. We obtain estimates of cortical depth for every fMRI voxel by using a distance map based upon a gray matter/white matter manual segmentation. This segmentation is derived from a combination of the mean image of reconstructed fMRI amplitude and unwrapped phase (equivalent to anatomical T2*weighted images, Duyn et al, 2007).
Results:
Replicating our earlier study, we found that pRFs are attracted towards the task location when averaged across all layers in V1. Furthermore, pRF attraction varied across cortical depth. Specifically, we observed that pRF attraction near the white matter border (i.e. deeper) was larger compared to attraction further away from the white matter border (i.e. more superficial).
Conclusions:
We found that spatial attention affects pRF position more at greater cortical depth in human V1. These results can be explained by a stronger effect of attention at this location in V1, where descending feedback connections terminate. These results extend earlier results on pRF attraction across different visual field maps and demonstrate that laminar imaging helps to bridge the gap between neurophysiology and functional imaging, indicating sites near the white matter border in V1 as the main target of endogenous attentional modulation.

Disclosures: **B.P. Klein:** None. **A. Fracasso:** None. **S.O. Dumoulin:** None.

Poster

432. Human Visual Cortex

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Topic: D.06. Vision

Title: Neural variability is an individual trait

Authors: *A. ARAZI[1], G. GONEN - YAACOVI[1], I. DINSTEIN[2];
[1]Dept. of brain and cognitive science, [2]Dept. of psychology, Ben Gurion Univ., Beer Sheva, Israel

Abstract: Introduction: The mammalian brain is an unstable system where neural responses to repeated presentations of an identical stimulus exhibit considerable trial-by-trial variability. Previous studies have reported that such neural variability is reduced (i.e., quenched) by the presentation of a stimulus, suggesting that the brain enforces stability when processing external stimuli. Does neural variability and variability quenching differ across individuals? Do some individuals exhibit reliably larger levels of variability than others? To address these questions we recorded neural responses with EEG from human subjects while they performed several cognitive and sensory tasks in two experimental sessions that were separated by a year. We quantified individual levels of trial-by-trial variability and variability quenching and assessed the reliability of between-subject differences across sessions and across the different tasks. **Methods:** Twenty four subjects completed two experimental sessions. Each session included four experiments: 1) Presentation of a checkerboard stimulus in the periphery while subjects performed an orthogonal color-detection task at fixation. 2) A choice reaction time task containing two visual stimuli. 3) A go-no-go response-inhibition task containing the same two visual stimuli. 4) A 2-back working memory task containing four visual stimuli. Neural responses were recorded with EEG and trial-by-trial neural variability was quantified before and after stimulus presentation to quantify neural variability and quenching in each subject. **Results:** Neural variability and variability quenching levels were positively correlated across experimental sessions and tasks ($0.65<r<0.93$, $p<0.01$). Furthermore, variability quenching was significantly smaller in the first visual task (where attention was diverted away from the checkerboard stimulus) in comparison to the other three tasks (where attention was focused on the stimulus). This demonstrated that attentional demand increased neural variability quenching, yet showed that between subject differences were much larger than those due to attentional modulation. **Conclusions:** The results revealed that trial-by-trial variability and variability quenching are stable individual traits that remain consistent regardless of the task being examined or the state of the subject.

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Poster

432. Human Visual Cortex

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ARC Centre for Excellence in Cognition and its Disorders Neural Markers Training Scheme

Title: Contextual modulation in human visual cortex: orientation tuning of surround suppression varies with the spatial extent of the surround

Authors: *S. WARDLE[1,2], K. SEYMOUR[1,2];
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Abstract: It is well-established that the response to visual stimuli placed within a neuron's receptive field can be modulated by stimuli placed in the surround regions outside the classical receptive field. Contextual modulation has also been demonstrated at the population level in human V1 using fMRI: the BOLD response to a central target grating is suppressed by the addition of a large annular surround grating. Surround suppression is orientation-tuned: greater suppression occurs when the target and surround gratings have the same orientation, and suppression is reduced when the target and surround gratings are orthogonal. Recent results in macaque V1 and human psychophysics suggest that the orientation-tuning of surround suppression is modulated by the spatial extent of the surround [Shushruth et al., 2013, J. Neuroscience]. Here we examine the spatial range of orientation-tuned surround suppression at the population level in human visual cortex with fMRI. In an optimized block design we presented target and surround gratings that varied both in their relative orientation (parallel or orthogonal) and importantly, in the spatial extent of the surround (near or far). We used independent localizer runs to isolate voxels in V1 that responded preferentially to the target. Consistent with previous results, greater suppression of the BOLD response to the target occurred for a parallel near surround than for an orthogonal near surround. However, the results differed for the far-surround, and facilitation of the BOLD response to the target occurred for the orthogonal far-surround. Our results in human V1 are consistent with previous findings in macaque V1 and human behavior suggesting differential orientation tuning of near and far surround suppression in early visual cortex.

Disclosures: **S. Wardle:** None. **K. Seymour:** None.

Poster

432. Human Visual Cortex

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 432.08/RR2

Topic: D.06. Vision

Support: NIH Grant N00014-14-1-0542

ONR Grant R01MH097320

Title: The phase of ongoing delta oscillations predicts eye movements and visually evoked responses in striate cortex during visual search

Authors: *N. N. THIGPEN;
Psychology: Behavioral and Cognitive Neurosci., Univ. of Florida, Gainesville, FL

Abstract: In recent animal work, oscillations in the delta range have been shown to align with the temporal structure of an attended stream of visual stimuli, suggesting delta oscillations may reflect a mechanism for attentional selection. Here, we investigate whether delta oscillations in the human visual cortex temporally align with eye movements and steady-state visual evoked potentials (SSVEP) during visual search. Thirty participants attempted to identify a hidden target in 80 natural scenes, while undergoing dense array EEG and eye tracking recordings. Each scene was luminance modulated at a rate of 30 Hz, to produce SSVEPs. SSVEP power was quantified as time-locked and phase-locked oscillations at the luminance-modulation frequency, using time frequency transforms of event-related EEG segments, whereas ongoing delta oscillations were quantified in the continuous data. At occipital electrode sites, delta oscillations were phase-coupled with eye movement onset, suggesting that delta phase alignment reflects a mechanism underlying oculomotor sampling of the environment during visual search. Delta oscillations were also phase-coupled with an increase in SSVEP power in the absence of an eye movement, suggesting visual information processing at the population level is facilitated during specific phases of the delta cycle. Taken together, these results suggest delta oscillations reflect a mechanism for active oculomotor and visual sampling of the environment during visual search behavior.

Disclosures: N.N. Thigpen: None.

Poster

432. Human Visual Cortex

Location: Halls B-H

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Program#/Poster#: 432.09/RR3

Topic: D.06. Vision

Support: ERC StG 2012_311751-BrainReadFBPredCode

Title: V1 & V2 receive high-level scene information via cortical feedback.

Authors: *A. T. MORGAN, L. S. PETRO, L. MUCKLI;
Inst. of Neurosci. & Psychology, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Introduction: Neurons in early visual cortex receive highly selective feedforward input that is amplified or disamplified by contextual feedback and lateral connections (Phillips, 2015). Many cortical areas feed back to early visual cortex, yet measuring feedback channels presents a central challenge to fully understanding neural computations (Muckli and Petro, 2013). To isolate feedback during scene processing we blocked feedforward input to subsections of human retinotopic visual cortex with a uniform visual occluder covering one quarter of the visual field (Smith and Muckli, 2010) while participants viewed 24 real-world scenes. We probed the information characteristics of feedback by selecting scenes spanning two abstract features that modulate V1 responses: scene category and depth (Kravitz, et al. 2011; Walther, et al. 2009). This allowed us to investigate whether feedback contains information about these features. **Methods:** We localized subsections of V1 and V2 in an fMRI experiment responding to either an occluded or non-occluded portion of the visual field, yielding four regions of interest: Occluded and Non-Occluded V1 and V2. We used multivariate pattern analyses (support vector machines [Smith and Muckli, 2010] and representational similarity analysis [RSA; Kriegeskorte, et al. 2008]) to compare and visualize scene response profiles in each region. **Results:** Using pattern classification techniques we found that response patterns in Occluded V1 and V2 contain individual scene, category and depth information. By testing classifiers on scenes withheld from training we found that category information was generalizable across scenes while depth information was not. Feedback to early visual cortex is therefore specific to individual scenes while concurrently conveying some high-level structure. Using RSA we show that Occluded V1 and V2 responses differed from each other, indicating that feedback to these two areas has unique information content. Additionally, Occluded and Non-Occluded V1 and V2 represented scenes differently than three popular biologically-inspired computational models (Weibull, Gist, and H-MAX-C2). **Conclusions:** Our results show that feedback to early visual cortex contains individual scenes, category and depth information. Moreover, feedback to V1 and V2 has unique information content. Additionally, these areas represent scenes differently from three popular biologically inspired computational models. Together these results highlight that a true

understanding of the neural computations of early visual cortex will involve understanding how and what information is conveyed by feedback.

Disclosures: **A.T. Morgan:** None. **L.S. Petro:** None. **L. Muckli:** None.

Poster

432. Human Visual Cortex

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Program#/Poster#: 432.10/RR4

Topic: D.06. Vision

Support: NIH Grant 1R24MH106096-01

Title: Array coils for ultra-high resolution columnar imaging in visual cortex

Authors: ***A. BECKETT**[1], A. T. VU[1,2], S. SCHILLACK[3], D. A. FEINBERG[1,2];
[1]Univ. of California, Berkeley, CA; [2]Advanced MRI Technologies, Sebastopol, CA; [3]Virtumed, Minneapolis, MN

Abstract: Innovations in hardware, such as the progression to ultra-high magnetic fields, and sequence development, i.e. accelerated image acquisition, have greatly increased the available resolution available for fMRI, and allowed the capability for the imaging of neural architecture at sub-millimeter resolutions. Specialized receiver hardware may be necessary to routinely allow sufficient SNR for this kind of high-resolution imaging. As part of our NIH BRAIN Initiative project (1R24MH106096-01), new coil designs have been developed to increase SNR specifically in the cortex, rather than more balanced SNR across both superficial and deep brain regions. We tested the utility of these new coils by focusing on a known fine-scale feature of human visual cortex: ocular dominance columns (ODCs).
ODC fMRI data were collected on a Siemens 7T scanner using a prototype coil: 8-channel receive, 1-channel transmit, with 4cm diameter loops in receiver array to optimize SNR in cortex. Data were collected using simultaneous multi-slice (SMS) EPI (resolution 0.5 mm isotopic, SMS-factor 2, in-plane IPAT 2, Matrix Size 180x160, 60 slices, 0% distance factor, flip angle 67, partial Fourier 5/8, TR=3000ms, TE=25.2ms, B/W=817 Hz, FOV 90x80.) The dedicated cortical imaging coil was combined with slice dithering for enhanced resolution (SLIDER), in which the slice thickness was doubled and slice positions overlapped to maintain closer slice spacing. This decreased aliasing and increased high spatial frequencies in the slice direction relative to 1mm isotropic voxels, with the benefit of increased SNR relative to 0.5mm slice thickness, leading to a net gain in sensitivity to ODCs.
Ocular dominance mapping was done using colored moving dot stimuli in three different colors:

red, green and yellow. Using custom anaglyph spectacles (using Kodak Wratten filters No. 25 (red) and 44A (cyan)) these dots stimulated either a single eye or both eyes simultaneously (Nasr 2016). Blank blocks (uniform black screen) were interspersed amongst the colored dot blocks. The use of the higher density coil array increased signal in peripheral brain regions, including cortex, , yielding increased tSNR and BOLD contrast at high resolution for 3D cortical fMRI. These gains allowed the potential for high-resolution, columnar scale imaging in timeframes much reduced than previous. The potential for high-resolution 3D columnar scale imaging in such a reduced time allows the potential to both localize and test the response properties of such features in a single scanning session, increasing the accuracy, reducing the need for combining data between sessions.

Disclosures: **A. Beckett:** A. Employment/Salary (full or part-time): Advanced MRI Technologies. **A.T. Vu:** A. Employment/Salary (full or part-time): Advanced MRI Technologies. **S. Schillack:** A. Employment/Salary (full or part-time): Virtumed LLC. **D.A. Feinberg:** A. Employment/Salary (full or part-time): Advanced MRI Technologies.

Poster

432. Human Visual Cortex

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 432.11/RR5

Topic: D.06. Vision

Title: Luminance modulates the contrast response in human visual cortex

Authors: *L. VINKE[1,2], S. LING[2,3,4];
[1]Grad. Program in Neurosci., [2]Ctr. for Systems Neurosci., [3]Psychological and Brain Sci., Boston Univ., Boston, MA; [4]Donders Inst. for Brain, Cognition, and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: The vast majority of models in vision downplay the importance of overall luminance in the neural coding of visual signals, placing emphasis instead on the coding of features such as relative contrast. Given that the visual system is tasked with encoding surfaces and objects in scenes, which often vary independently in luminance and contrast, it seems likely that luminance information is indeed encoded and plays an influential role in visuocortical processing. However, the cortical response properties that support luminance encoding remain poorly understood. In this study, we investigate the interaction between contrast response and luminance in human visual cortex, using fMRI. We assessed BOLD responses in early visual cortex (V1-V3) while participants viewed checkerboard stimuli that varied in contrast and luminance. Specifically, we utilized an adaptation paradigm that allowed us to reliably measure contrast responses at multiple

spatial scales (voxel-wise and retinotopic), and across a set of luminance levels. To control for changes in pupil diameter with varying luminance levels, stimuli were viewed monocularly through an artificial pupil. We found that the extent to which the overall luminance of a signal modulates responses in visual cortex is contrast dependent, with reliable increases in responses along with increasing luminance levels, but only occurring at low levels of contrast. These results reveal that the visuocortical neural code does indeed retain and utilize information about the luminance of a visual signal, but appears to preferentially modulate the response only at low-to-zero contrast levels. This finding suggests that luminance likely plays a dominant role in visual tasks such as our perceptual encoding and segregation of surfaces.

Disclosures: **L. Vinke:** None. **S. Ling:** None.

Poster

432. Human Visual Cortex

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Topic: D.06. Vision

Support: NWO 406-14-085

Title: Cortical depth dependent 7T BOLD responses to parametrically varied luminance contrast

Authors: ***I. MARQUARDT**, M. SCHNEIDER, O. GULBAN, D. IVANOV, K. ULUDAG; Dept. of Cognitive Neurosci., Maastricht, Netherlands

Abstract: Introduction
High-resolution functional MRI (fMRI) studies at high field strength (7 Tesla) have demonstrated the feasibility of sampling functional signals at different cortical depth levels [1, 3, 5, 6, 7, 8]. However, laminar specificity of the neuronal response is degraded by the hemodynamic response. Here, we demonstrate that cortical depth specificity in V1 can be recovered by measuring responses to parametrically varied luminance contrast levels of a grating stimulus.
Methods
Human participants (n=3) were presented with visual grating stimuli at four different luminance contrasts. Retinotopic mapping was performed using population receptive field estimation, allowing delineation of V1 [2]. Functional MRI data were acquired on a 7 Tesla scanner using a gradient-echo EPI sequence (resolution 0.7 mm isotropic). We also acquired T1 images with identical distortions using a multi-inversion time inversion recovery sequence, allowing for an unbiased sampling of the cortical ribbon [4].

Results

Figure 1A shows the mean percent signal change (PSC) change at each depth level for each stimulus luminance contrast averaged across all trials in all participants. A two-way AVNOVA showed a significant effect of stimulus luminance contrast ($F(3, 5148) = 272.9$, $p < 0.001$) and cortical depth level ($F(8, 5148) = 67.4$, $p < 0.001$), as well as an interaction ($F(24, 5148) = p < 0.001$). As expected, stronger stimulus luminance contrast led to a stronger PSC at all cortical depth levels (Figure 1B).

Conclusion

Our results demonstrate that high-resolution fMRI at 7T in conjunction with a parametric experimental design can detect differences in the response properties across cortical depths in human participants.

[1] De Martino et al (2013) PLoS ONE 8(3) e60514.
[2] Dumoulin & Wandell (2008) Neuroimage 39, 647-660.
[3] Huber et al (2015) NeuroImage 107, 23-33
[4] Kashyap et al (2016) ISMRM 2016
[5] Koopmans et al (2010) Human Brain Mapping 31(9), 1297-1304
[6] Koopmans et al (2011) NeuroImage 56(3), 1276-1285
[7] Olman et al (2012) PLoS ONE 7(3) e32536
[8] Polimeni et al (2010) NeuroImage 52(4), 1334-1346

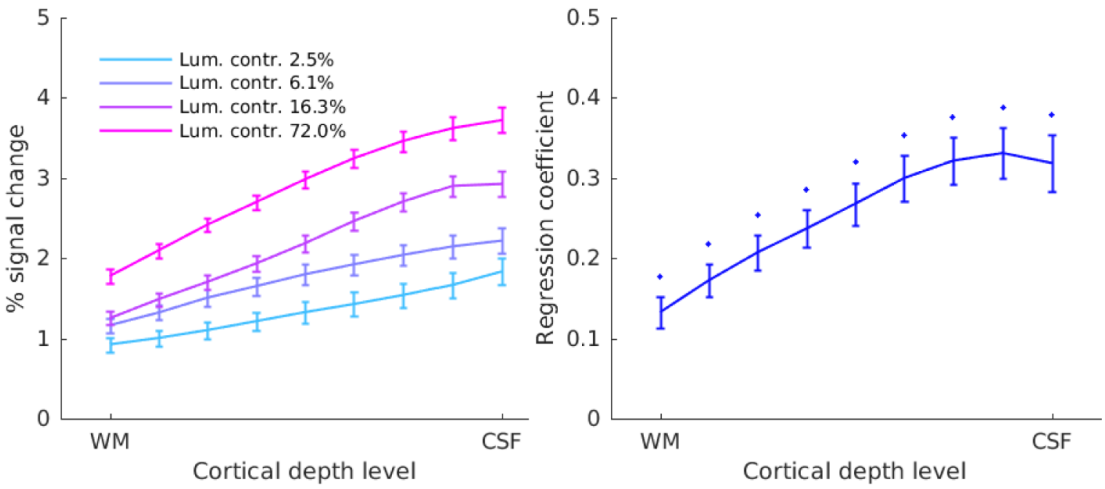

Figure 1. (A) Percent signal change (PSC) for each of nine cortical depth levels, within a region of interest (ROI) in primary visual cortexthat was responsive to the experimental stimulus. (B) Regressioncoefficients for each of nine cortical depth levels. We regressed thePSC of each trial on stimulus luminance level, separately for eachcortical depth level, using a linear model. The resulting regressioncoefficient indicates how strongly the response of voxels at therespective depth level was modulated by increasing stimulus luminancecontrast. (Error bars represent the standard error of the mean, asterisks indicate significance of regression model at p(bonf.) < 0.001)

Disclosures: I. Marquardt: None. **M. Schneider:** None. **O. Gulban:** None. **D. Ivanov:** None. **K. Uludag:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: NIH Pioneer (1DP1NS083063-01)

HHMI

Title: Extra-classical receptive field effects on visual processing in the awake rodent

Authors: *F. LUONGO, L. LIU, D. TSAO;
Caltech, Pasadena, CA

Abstract: Traditionally, methods for describing the responsiveness of visual neurons in the mouse have centered around responses within a neuron's receptive field. However, neurons are often modulated by context outside of this traditional receptive field also termed the extra-classical receptive field. Such contextual modulation e.g. figure-ground and border ownership modulation provide valuable insights into the underlying computational machinery that the brain uses to segment and identify discrete objects in the visual world. Determining to what extent some of these computations in the extra-classical receptive field are conserved across animals is an important step in establishing the strength and limitations of the rodent as a model of visual computation. We presented a battery of figure ground and border ownership stimuli to rodent's while observing the activity of ~100 neurons simultaneously using 2-photon calcium imaging. We relate the activity of these neurons to modulation that occurs both within and outside the traditional receptive field. Neurons show varied responses to stimuli and exhibit contextual modulation outside of the traditional receptive field. We observe these responses in both primary and extra-striate visual areas as defined using widefield calcium imaging. This work lays the foundation for understanding the types of computations performed in the rodent visual hierarchy and its relation to previous work in cat and primate models.

Disclosures: **F. Luongo:** None. **L. Liu:** None. **D. Tsao:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: NIH Grant EY019288

Pew Charitable trusts

Human Frontier Science Program

Title: Binocular integration in mouse using stereoscopic cues to guide behavior

Authors: *V. CHOI[1], S. JOO[2], J. M. SAMONDS[1], A. C. HUK[2], N. J. PRIEBE[1];
[1]Dept. of Neuroscience, Ctr. for Perceptual Systems, Ctr. for Learning a, [2]Dept. of Neurosci. and Psychology, Ctr. for Perceptual Systems, Univ. of Texas at Austin, Austin, TX

Abstract: Despite the presence of disparity selective neurons in mouse primary visual cortex (Scholl et al. 2013), there has been little evidence that mice integrate binocular cues to guide behavior. Previous work has demonstrated that rodents can estimate the size of a gap (Legg & Lambert, 1990; Kerr et al., 2013), but it is unclear whether the binocular or monocular depth cues were used. To determine whether binocular cues can drive behavior, we trained animals to distinguish between toward and away motion in depth. Drifting vertical gratings were presented to each eye using a stereoscopic projector and mice were trained to stop for the toward condition and walk for other conditions. "Toward" stimuli are generated by presenting the left eye with a rightward grating and the right eye with a leftward grating; “away” stimuli are generated by presenting the left eye with a leftward grating and the right eye with a rightward grating. We also presented frontoparallel motion in which the gratings moved same direction. Trained mice walked significantly faster for the away and frontoparallel conditions than the toward condition. The behavior depended critically on the binocular depth cues we employed with our stereoscopic projector: the difference in walking speed was abolished when the polarizing glasses used for stereoscopic stimulation were removed and walking speed for toward and away conditions reversed when the position of the polarized glasses are swapped. To determine whether activity in primary visual cortex (V1) impacts this behavior we inactivated V1 using optogenetics while animals performed the motion in depth task. Inactivating V1 eliminates the difference in walking speed for toward and away conditions. These results indicate that binocular cues are used to guide behavior in mice, as in other mammals, and that signals in V1 are essential for the expression of this behavior.

Disclosures: **V. Choi:** None. **S. Joo:** None. **J.M. Samonds:** None. **A.C. Huk:** None. **N.J. Priebe:** None.

Poster

433. Rodent Visual Cortex

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Support: Simons Collaboration on the Global Brain Fellowship 349431

Simons Collaboration on the Global Brain Award 325295

NIH Grant R01 EY02874

RPB Stein Innovator Award to M.P. Stryker

Title: Locomotion enhances information represented in mouse visual cortex both by increasing firing rates and decreasing correlations

Authors: ***M. C. DADARLAT**, M. P. STRYKER;
Physiol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Locomotion is a behavioral state that increases stimulus-evoked responses in mouse primary visual cortex (V1); the response changes have previously been characterized as changes in gain. But spikes cost metabolic energy; is something purchased at this price? As more visual information is available to an animal moving through the environment, perhaps locomotion enhances the information represented in the visual system. To test this hypothesis, we made simultaneous extracellular recordings from 40-83 single neurons in the primary visual cortex of each of 6 awake mice that were presented with moving gratings in the monocular visual field contralateral to the recording site. Mice were free to run or stand stationary on a spherical treadmill floating on an air stream, and we monitored locomotion speed and heading direction. We found that V1 does encode information more accurately during locomotion, and not merely by way of increased firing rates. Locomotion-induced increase in firing rates enhanced the "stimulus-specific information" of individual neurons, as well the population-based discriminability of stimuli. Furthermore, discriminability was improved even when population firing rates did not change with locomotion because locomotion also decreased noise correlations across the population. These improvements were both realized by a particular type of neuron: broad-spiking presumptive excitatory neurons with high multiplicative gain. Together, these

results suggest a computational function for locomotion-induced modulation in neural firing and explain how this function is achieved.

Disclosures: **M.C. Dadarlat:** None. **M.P. Stryker:** None.

Poster

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Support: NIH Grant R01EY020950

Title: Thalamocortical and intracortical contributions to binocular matching of orientation preference in mouse visual cortex

Authors: ***Y. GU**, J. CANG;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: Binocular neurons in the visual cortex are tuned to similar orientations through the two eyes. In mice, the developmental process that gives rise to binocularly matched tuning takes place in a critical period in early life (approximately postnatal day 20-30, Wang et al., 2010 and 2013). In this study, we used in vivo whole cell recording in urethane-anesthetized mice to investigate the synaptic and circuit mechanisms of binocular matching.
First, we found that in adult mice, the subthreshold membrane potential (Vm) responses of individual layer 4 cortical neurons are binocularly matched in their orientation preference to drifting sinusoidal gratings (the difference in preferred orientation through the two eyes: ΔO = 21.1±3.2°, n= 14 cells). In contrast, in juvenile mice (P15-21) before the critical period, the orientation preference of Vm responses are mismatched between the two eyes (ΔO = 50.5±3.8°, n = 25), consistent with our previous studies of spiking responses (Wang et al., 2013).
Next, by comparing visually-evoked excitation under voltage clamp between before and after optogenetically silencing visual cortex, we were able to isolate thalamocortical and intracortical excitatory input to individual layer 4 neurons. In adult mice, we found that both thalamic and cortical excitatory inputs are tuned to similar orientations through the two eyes, leading to binocularly-matched total excitation (thalamic ΔO = 19.4±5.5°, cortical ΔO = 17.2±3.2°, total ΔO = 20.6±4.3°, n = 15). Furthermore, for each eye, the thalamic input predicts the orientation tuning of cortical excitatory inputs (the orientation difference between thalamic and cortical tuning: ΔO = 17.0±4.4° for contra response, and ΔO = 24.4±6.0° for ipsi responses, n = 15). This result is consistent with the recent studies that used similar techniques for contralateral responses

and supported the feed-forward model of thalamocortical transformation (Lien and Scanziani, 2013; Li et al., 2013). In young mice before the critical period (P15-21), on the other hand, both thalamic and cortical excitation onto layer 4 neurons are mismatched in their orientation tuning. Interestingly, the thalamic input appears to be slightly better matched binocularly than the cortical input (thalamic $\Delta O = 35.9 \pm 6.3°$, cortical $\Delta O = 49.4 \pm 6.6°$, $n = 15$). Further experiments and analysis are ongoing to investigate the relative contributions of thalamocortical and intracortical circuits in the matching process. Together, our experiments will begin to reveal the synaptic and circuit mechanisms of how critical period plasticity matches eye-specific inputs in the cortex to achieve normal binocular vision.

Disclosures: **Y. Gu:** None. **J. Cang:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: CIHR-MOP111003

NSERC-238835-2011

Title: Basal forebrain projections to different rat visual cortical areas

Authors: F. HUPPÉ-GOURGUES, K. JEGOUIC, S. BOUHABEL, *E. H. VAUCHER; Univ. of Montreal, Montreal, QC, Canada

Abstract: Acetylcholine is an important neurotransmitter for the regulation of the visual attention, plasticity and perceptual learning. The acetylcholine is released in the visual cortex predominantly by the cholinergic fibers projecting from the basal forebrain. The stimulation of this region may provide a beneficial potentiation of visual processes. However, little is known about the topographical organization of these basalocortical projections, i.e. if there are fine topographical projections from the different basal forebrain nuclei to the primary and secondary visual cortical areas. The aim of this study is to map these basalocortical projections. Small injections of retrograde tracers (fluorescent cholera toxin b fragment) in different quadrants of the primary and secondary visual cortex of long Evans rats were performed (n=8). The retrogradely labelled cell bodies in the nucleus basalis, substantia innominanta and diagonal band of Broca were mapped ex-vivo with a computer-assisted microscope stage controlled by stereological software suite (Microbrightfield). Choline acetyltranferase immunohistochemistry

was used to identify cholinergic cells. Our results show a predominance of projections coming from the horizontal band of Broca's neurons. The projections were not clearly topographically organized, each location of the nucleus innervating every cortical areas observed. Our preliminary results confirm that these cells are cholinergic. The absence of a clear topography of the horizontal band of Broca projections to the visual cortex opens the possibility to stimulate a large cortical region upon stimulation of the cholinergic cells of the horizontal limb of the diagonal band of Broca.

Disclosures: F. Huppé-Gourgues: None. **K. Jegouic:** None. **S. Bouhabel:** None. **E.H. Vaucher:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: BBSRC Grant BB/K001817/1

Royal Society Industry Fellowship

Title: The entropy of neural ensemble firing patterns in mouse primary visual cortex correlates with behavioural performance

Authors: *M. TOLKIEHN, A. BERDITCHEVSKAIA, S. R. SCHULTZ;
Imperial Col. London, London, United Kingdom

Abstract: Simultaneous in-vivo electrophysiological recordings in populations of neurons are a widespread technique to investigate neural activity. To this end there has been much research into single cell properties and their link to behavioural outcome, whilst the contribution of statistical properties of cell assembly firings has been neglected. A prominent model to examine this is mouse primary visual cortex (V1), as it is located at an early cortical processing stage, allowing us to relate neural activity to the stimulus more directly.
To investigate how simultaneously recorded activity patterns relate to task performance, we trained head-fixed, water-restricted mice to respond to vertical and horizontal moving gratings, which were associated with a positive reinforcement (water supplement) or a punishment (air puff to flank). This paradigm required the mouse to initiate a response (lick) or to withhold a lick. Analysis of Multi-Unit-Activity (MUA) recorded by in-vivo multi-shank multi-laminar electrophysiology in V1 of the awake behaving animal indicated a correlation between task

performance and spatial pattern distribution. In particular, the results reveal a negative correlation between (spatial) pattern entropy and the animal's task performance. In addition, response clusters for correct responses appear closer in high performance regimes, particularly for correct decisions (True Positive, True Negative). This means the pattern dissimilarity, estimated with Jensen-Shannon Divergence, decreases at higher accuracy levels. Independent Poisson surrogate data are able to capture this to an extent, but fail to fully explain this relation, which suggests this relationship cannot be explained by firing rate differences alone. The entropies in surrogate data seem to slightly exceed those of the real data ($p<=0.001$ signed rank), and the JS divergences of the surrogate data consistently underestimate those of real data ($p<0.001$, signed rank). To understand what additional features may explain this relationship, we fit (Energy-based) models that allocate a probability to each observed spatial firing pattern. Our results show that including couplings between sites increases the likelihood of our data over the independent model by around 0.2 bit/spike, while also decreasing the divergence between empirical and model distribution. This suggests that laminar interactions in V1 may play a role in behavioural performance.

Disclosures: **M. Tolkiehn:** None. **A. Berditchevskaia:** None. **S.R. Schultz:** None.

Poster

433. Rodent Visual Cortex

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RIKEN-BSI Research Grant

Title: Sculpting the dynamics of neuronal networks in the mouse cortex with optogenetic tools

Authors: T. TSUBOTA, D. LYAMZIN, *A. BENUCCI;
RIKEN Brain Sci. Inst., Wako City, Saitama, Japan

Abstract: The fine tuning of the connectivity patterns among neurons is what confers upon them the computational power needed to perform complex operations. However, the biological processes underlying such changes are not fully understood. Spike-timing dependent plasticity (STDP) has been hypothesized to play a key role, but whether or not and to what extent STDP can indeed change the functional architecture of cortical networks in adult animals is largely unknown.

Here we used an in-vivo preparation for simultaneous optogenetic stimulation and two-photon imaging of neuronal populations in awake mice. We expressed a red-shifted channelrhodopsin (ChrimsonR) in excitatory neurons in the dorsal cortex of mice (n=3 animals). We then used a digital micro-mirror device (DMD) for spatially patterned excitation of ChrimsonR-expressing cells. We paired DMD stimulation of an individual cell (the driver) with a delayed stimulation (10ms) of several tens of nearby cells (the followers) to induce driver-followers STDP. Using two-photon GCaMP imaging we studied the population dynamics of the followers after the driver's firing, either induced by optogenetic stimulation or occurring during spontaneous activations, and compared the neural dynamics before and after the pairing protocol.
We found that the responses of the followers (n=128 cells) changed in a complex fashion. After paired stimulation, the correlation strength with the driver increased in some cells (45%), at various time-lags, while it decreased in others (33%). Other neurons (22%) were mostly unaffected. To understand this heterogeneity at the network level, we studied the population responses in a reduced dynamical space using principal-component analysis (PCA). In this representation, the network dynamics showed consistent changes across animals; the dynamics of the followers became closer to that of the driver cell after optogenetic pairing. Strikingly, these effects persisted also for a few hundreds of milliseconds after spontaneous firings of the driver.
In conclusion, our methodology provides an effective in-vivo experimental tool to study activity-dependent functional changes in cortical networks of adult animals. Preliminary results demonstrate that although changes induced by paired optogenetic stimulation are diverse at the level of single cells, the overall population dynamics has a tendency to be "attracted" toward the dynamics of the driver cell. Such optogenetic-induced changes might reflect a STDP-based dynamic mechanism of synaptic-weight scaling aiming to efficiently process information in view of novel behavioral demands.

Disclosures: **T. Tsubota:** None. **D. Lyamzin:** None. **A. Benucci:** None.

Poster

433. Rodent Visual Cortex

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Program#/Poster#: 433.08/RR14

Topic: D.06. Vision

Title: Morphologically-defined cell types in mouse primary visual cortex

Authors: ***S. A. SORENSEN**, T. DESTA, A. HENRY, R. DALLEY, D. SANDMAN, N. THATRA, G. WILLIAMS, J. BERG, X. LIU, K. GODFREY, D. FENG, N. GOUWENS, C.

LEE, Z. ZHOU, H. PENG, Y. WANG, A. BERNARD, L. NG, J. HARRIS, H. ZENG;
Neurosci., Allen Inst., Seattle, WA

Abstract: In an effort to understand the full diversity of cell types in mouse primary visual cortex (V1), the Allen Institute for Brain Science established an in vitro single cell characterization pipeline, designed for the collection of electrical and morphological properties from 100s -1000s of neurons.
Selectivity and coverage across cell types has been maximized by targeting both Cre-positive and Cre-negative neurons for patch recordings and biocytin labeling in layer- and interneuron-selective Cre mouse lines. A subset of neurons with electrical recordings were reconstructed in 3D. Dendrite only reconstructions were prioritized for spiny, pyramidal neurons, while full reconstructions of both dendrites and local axon were prioritized for aspiny and sparsely spiny interneurons. Both qualitative and quantitative approaches have been pursued to understand the range of morphological types that exist in V1. Qualitative analyses provide a benchmark dataset to which more rigorous quantitative analyses can be compared. Thus far most of the previously described rodent V1 types have been observed in our dataset. Morphological types are also compared to quantitatively identified electrical types. For particularly unique types, such as neurogliaform cells, we see very good correspondence, and in most cases electrical types support morphological types. Recently we have also begun comparing morpho-electrical types to transcriptomically-defined types (Tasic et al., Nature Neuroscience, 2016). Together these analyses will be used to establish a comprehensive, multimodal taxonomy of neurons in mouse V1. These data are all made publicly available on our Allen Institute Cell Types Database (www.brain-map.org).
Nat Neurosci. 2016 Feb;19(2):335-46. doi: 10.1038/nn.4216. Epub 2016 Jan 4.

Disclosures: **S.A. Sorensen:** None. **T. Desta:** None. **A. Henry:** None. **R. Dalley:** None. **D. Sandman:** None. **N. Thatra:** None. **G. Williams:** None. **J. Berg:** None. **X. Liu:** None. **K. Godfrey:** None. **D. Feng:** None. **N. Gouwens:** None. **C. Lee:** None. **Z. Zhou:** None. **H. Peng:** None. **Y. Wang:** None. **A. Bernard:** None. **L. Ng:** None. **J. Harris:** None. **H. Zeng:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: 1R01MH101198-01

R01 MH105427 A1

5 F31 EY025185-02

Title: Behavioral state modulates 3-5 Hz membrane potential oscillations in mouse visual cortex.

Authors: *M. EINSTEIN[1], P.-O. POLACK[3], P. GOLSHANI[2];
[1]Neurol., UCLA, Playa Del Rey, CA; [2]Neurol., UCLA, Los Angeles, CA; [3]Rutgers Univ., Newark, NJ

Abstract: 3-5 Hz oscillations in visual cortex have been implicated as important during reward expectation and working memory. Here, we demonstrate the underlying membrane potential changes that drive local field potential changes in primary visual cortex (V1) during 3-5 Hz oscillations by measuring the membrane potential (Vm) from V1 L2/3 excitatory, PV+, and SOM+ neurons of awake and behaving mice. During oscillation epochs, excitatory neurons reduced their spontaneous and visually evoked firing rate while PV+ and SOM+ inhibitory neurons fired rhythmically with each cycle of the oscillation. 3-5 Hz oscillation timing was also drastically different when animals were passively viewing stimuli versus when they engaged in a visual discrimination task. But, when animals performed a similar non-visual discrimination task, oscillations were rare. Altogether, our results indicate that 3-5 Hz oscillations decrease the gain of excitatory neurons, and oscillation recruitment relies on both visual stimulation and an animal's internal state. Our findings suggest that 3-5 Hz oscillations may act as a mechanism to decrease visually evoked L2/3 cortical neuron firing rates, potentially to prevent interference of the sensory driven responses with internally generated representations.

Disclosures: **M. Einstein:** None. **P. Polack:** None. **P. Golshani:** None.

Poster

433. Rodent Visual Cortex

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RIKEN-BSI Research Grant

Title: Invariant and abstract perceptual representations in mouse decision-making

Authors: *R. AOKI, D. LYAMZIN, M. ABDOLRAHMANI, M. J. MORAIS, A. BENUCCI; RIKEN Brain Sci. Inst., Wako City, Saitama, Japan

Abstract: The ability to form abstract perceptual representations that are invariant to physical transformations of sensory stimuli (e.g. size, luminance, etc) is a hallmark of primate high-level cognition. Such ability is critical to efficiently interact with a continuously changing natural environment, but little is known about its neural basis.
To study this problem, we trained mice in a visual decision-making task that required the animal's ability to form an abstract and invariant representation of a stimulus' orientation. More specifically, in a two-alternative forced choice task, the animal had to choose which of two grating stimuli was more vertical. Crucially, neither of the orientations were vertical.
To train the mice in this complex task, we used a fully-automated training setup for voluntary head fixation which allowed us to train ~30 mice/day (10^4 trials/day). During the time course of 3-4 weeks, mice increased their performance (n = 4 mice), with evidence for both procedural and perceptual learning, as quantified by a decrease in lapse rate (84% ± 6%, $p < 0.05$) and bias (74% ± 9 %, $p < 0.05$), and an increase in sensitivity (76% ± 19 %, $p < 0.05$) of the psychometric curves from naïve to expert animals (> 85% correct rate).
To verify the hypothesis that mice solved this task by resorting to an abstract and invariant representation of the target orientation, we demonstrated that mice did not utilize low-level features e.g. local luminance or contrast, nor memorized the luminance patterns. Moreover, the performance remained unchanged when introducing novel orientations or when changing the spatial frequency of the gratings. Finally, mice trained with a horizontal target orientation performed equally well.
Taking advantage of our automated setup for voluntary head fixation, we are combining behavioral observations with wide-field GCaMP imaging of all 10 retinotopically identified visual areas. Preliminary results reveal a significant correlation between the GCaMP signal and several learned task-related events.
In conclusion, our results strongly constrain the pool of possible functional principles underlying this cognitive task, and suggest a more fundamental, robust mechanism that can be conserved even across dramatic differences in putative neural resources, as evident in view of the three orders of magnitude fewer neurons in the mouse relative to the primate brain.

Disclosures: R. Aoki: None. **D. Lyamzin:** None. **M. Abdolrahmani:** None. **M.J. Morais:** None. **A. Benucci:** None.

Poster

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Support: NEI (DP1EY024503, R01EY011787)

DARPA SIMPLEX N66001-15-C-4032

U. S. Army Research Office under contract number W911NF-12-1-0594 (MURI)

Title: Identification of neuronal ensembles from primary visual cortex *In vivo* using probabilistic graphical models

Authors: *S. HAN[1], L. CARRILLO-REID[1], E. TARALOVA[1], T. JEBARA[2], R. YUSTE[1]; [1]Biol. Sci., [2]Computer Sci., Columbia Univ., New York, NY

Abstract: The coordinated firing of neuronal populations is considered to be the substrate of sensory, behavioral and cognitive functions. In particular, neuronal ensembles in primary visual cortex represent specific features of visual stimuli. However, how the functional connectivity in local microcircuits relate to their function has been difficult to elucidate *in vivo*. The development of recording and stimulation techniques with single cell precision has opened the possibility of testing the function and computation principles of cortical ensembles during physiological processes. To address these questions, it is necessary to identify online the most representative elements from a given neuronal ensemble. Here we used two-photon calcium imaging to record the responses to drifting gratings of layer 2/3 neurons in primary visual cortex of awake behaving mice, and then applied probabilistic graphical models to find the most representative neurons from each cortical ensemble. We demonstrate that the activity of the most representative neurons identified from each cortical ensemble is sufficient to predict a given visual stimuli. We anticipate that our approach will allow the design of close-loop two-photon optogenetic experiments with single cell resolution to test the physiological role of cortical ensembles during behavioral tasks.

Disclosures: S. Han: None. **L. Carrillo-Reid:** None. **E. Taralova:** None. **T. Jebara:** None. **R. Yuste:** None.

Poster

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Topic: D.06. Vision

Support: NIH Institutional Grant T32-NS058280

NIH Grant EY023871-02

Title: Cholinergic modulation of an inhibitory microcircuit changes dendritic integration after the visual critical period

Authors: *C. E. YAEGER, K. L. KWAN, J. T. TRACHTENBERG;
Neurobio., UCLA, Los Angeles, CA

Abstract: We find that changes in cholinergic input to somatostatin-expressing (SST) interneurons alters dendritic encoding of layer 5 excitatory neurons. Using fast resonant scanning two-photon microscopy in combination with genetically-encoded calcium indicators, layer 2/3 inhibitory interneurons and apical dendrites of layer 5 excitatory neurons were imaged in the primary visual cortex of awake, behaving mice during the visual critical period (p28) and after critical period closure (p60). While SST cells responded to visual stimulation in both groups of awake behaving mice, there was a divergence in SST response between age groups during locomotive events: during the critical period, SST cells are active during movement, but in adults, SST cells are suppressed. These findings were corroborated with slice recordings of SST interneurons and cholinergic stimulation. Furthermore, SST cells target layer 5 pyramidal cell apical dendrites, and local dendritic calcium events were also altered depending on the age and activity of the mouse: during the presence of greater inhibition from SST interneurons, more local dendritic calcium events occurred. This age-dependent shift in dendritic coding is expected to contribute to the large-scale plasticity characteristic of the juvenile cortex.

Disclosures: C.E. Yaeger: None. **K.L. Kwan:** None. **J.T. Trachtenberg:** None.

Poster

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Support: Centre for Integrative Neuroscience (DFG Exec 307)

European Research Council Starting Independent Researcher Grant project PERCEPT

LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative

Title: PV interneurons in visual cortex control contrast sensitivity and spatial integration of pyramidal cells

Authors: *M. FIORINI[1,2,3], S. ERISKEN[1,2,3], A. VAICELIUNAITE[1], O. JURJUT[1], S. KATZNER[1], L. BUSSE[1,3];
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Abstract: In primary visual cortex (V1), the largest neuronal population mediating GABAergic inhibition is represented by fast-spiking parvalbumin-positive interneurons (PV+ INs). Previous studies suggested that PV+ INs influence low-level visual processing by controlling contrast gain and shaping spatial integration of pyramidal cells. Interestingly, altered contrast perception and spatial integration are associated with schizophrenia, where one theory proposes that hypofunctioning NMDA-glutamate receptors (NMDAR) might cause deficient excitatory drive to PV+ INs (Marin, 2012). Following this model, we investigated whether selective ablation of NMDAR in PV+ INs was sufficient to alter sensory processing at early stages of the visual pathway.
We crossed PV-Cre mice with mice carrying floxed alleles of the obligatory NMDAR NR1 subunit and compared visual responses between transgenic (NR1-PVCre$^{-/-}$) and control animals. We recorded extracellular single unit activity from V1 and dorsolateral-geniculate nucleus (dLGN) of head-fixed mice placed on a spherical treadmill. For the analysis of neural activity, we concentrated on periods in which animals were stationary. Based on spike wave-shapes, we classified V1 neurons as broad- or narrow-spiking.
To assess how the reduction of NMDAR-mediated PV+ excitation changes baseline response properties in the V1 population, we first considered spontaneous activity. As expected, NMDAR ablation in PV+ INs led to lower firing rates in narrow-spiking and higher firing rates in broad-spiking neurons. We then restricted our analyses to broad-spiking, putative excitatory neurons and probed the role of NMDAR in PV+ INs for spatial integration: we observed that receptive field center sizes of V1 cells in mutants were smaller as compared to controls, and suppression strength was increased. Since it is well known that spatial integration depends on stimulus contrast, we tested the hypothesis that NMDAR dysfunction in PV+ INs could contribute to the processing of contrast. We found that broad-spiking V1 cells in NR1-PVCre$^{-/-}$ mice had significantly higher contrast sensitivity across all cortical layers as compared to controls. This increase in sensitivity seems to be a cortical phenomenon as contrast responses were similar between genotypes for neurons in dLGN.
Together, our results demonstrate a critical role of PV+ INs for shaping contrast sensitivity and spatial integration of pyramidal cells. We speculate that reduced glutamatergic excitation of cortical PV+ INs increases the effective stimulus drive in the V1 network, which in turn translates into a regime of more focused spatial integration.

Disclosures: **M. Fiorini:** None. **S. Erisken:** None. **A. Vaiceliunaite:** None. **O. Jurjut:** None. **S. Katzner:** None. **L. Busse:** None.

Poster

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Searle Foundation

Title: 2-photon imaging of task-dependent cortical population dynamics during a visual discrimination

Authors: *J. B. WEKSELBLATT, R. D. DI RICCO, C. M. NIELL;
Biol., Univ. of Oregon, Eugene, OR

Abstract: Neuronal responses in primary visual cortex (V1) are modulated by behavioral relevance. We sought to understand how information about specific features relevant to a task are encoded across populations of neurons. We therefore performed recordings of activity evoked by identical visual stimuli while mice performed one of two visual discrimination tasks (an orientation discrimination or a spatial location judgment) as well as during passive viewing of these same stimuli. 2-photon imaging using a transgenic mouse, expressing GCaMP6s in excitatory neurons throughout cortex allowed us to measure activity in hundreds of neurons simultaneously in order to extract the tuning properties of individual neurons as well as the dynamics of large ensembles on individual trials.
In these experiments, head-fixed mice were trained on a 2-alternative forced choice behavior to report either the orientation or location of a square-wave grating patch, using their direction of locomotion on a freely rotating track ball. Widefield imaging showed dramatic changes in mesoscopic response patterns over the course of learning the task. Once mice achieved a threshold level of performance, we imaged responses of hundreds of simultaneously recorded neurons in the functionally defined region of V1 representing the stimulus locations, during the behavioral tasks and during passive viewing of the same stimuli. Neurons in layer 2/3 of visual cortex show diverse coding properties including both classical orientation selective responses as well as non-specific suppression of responses based on retinotopic location. Analysis of population-wide encoding revealed dramatic shifts in large-scale dynamics with engagement in specific tasks. These findings extend our understanding of how encoding of visual stimulus

properties is integrated with task-related cortical dynamics.
This work was supported by NIH T32 HD007348 and F31 EY025459 (J.W.), NIH DP2 843 EY023190, NIH R01 EY023337, and the Searle Foundation (C.M.N.).

Disclosures: J.B. Wekselblatt: None. **R.D. Di Ricco:** None. **C.M. Niell:** None.

Poster

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Allen Institute for Brain Science

Title: Neuromodulatory axon activity in the visual cortex

Authors: *R. S. LARSEN, J. ZHUANG, D. OLLERENSHAW, T. DAIGLE, J. WATERS; Neural Coding, Allen Inst. For Brain Sci., Seattle, WA

Abstract: Visual cortical neuron responses are strongly influenced by behavioral state. Neuromodulatory projections from subcortical nuclei have been hypothesized to be important in modulating cortical responses to various behavioral states, but how and when these systems influence the visual cortex is just beginning to be understood. Previous studies have demonstrated that cholinergic, noradrenergic, and serotonergic projections innervate the visual cortex. To study the influence of these projections during visually-guided behavior, we sought to achieve widespread expression of the genetically-encoded calcium sensor GCaMP6 in neuromodulatory axons which innervate the visual cortex. We found that reporter mice expressing Cre-dependent GCaMP6 from the ROSA-26 allele drove only weak expression of GCaMP6 in neuromodulatory axons in the visual cortex. Similarly, triple transgenic mice expressing a neuromodulatory Cre, Cre-dependent tTA, and a tetO promoter-driven GCaMP6 from separate alleles failed to express any GCaMP6 in the somata of some neuromodulatory cell types. In contrast, recently developed Ai148 (TIT2L-GCaMP6f-tTA2) reporter mice which drive both Cre-dependent GCaMP6 and tTA expression from the TIGRE locus resulted in enhanced GCaMP6 labeling in neuromodulatory axon types when these reporter mice were crossed to neuromodulatory Cre lines, such as Chat-IRES-Cre. We have utilized these new tools to correlate mouse behaviors with the activity of neuromodulatory axons both across cortical areas using widefield imaging, and with cellular resolution using *in vivo* 2-photon imaging of the

cortex of awake mice. By imaging the activity of neuromodulatory projections into the visual cortex during behavior, we aim to determine mechanisms for how the cortex modulated by changes in behavioral state.

Disclosures: **R.S. Larsen:** None. **J. Zhuang:** None. **D. Ollerenshaw:** None. **T. Daigle:** None. **J. Waters:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Title: Robust electro-physiological type identification using co-clustering analysis of clustering tree

Authors: ***C. LEE**[1], N. GOUWENS[1], K. LEPAGE[1], V. MENON[1], T. BAKKEN[2], S. SUNKIN[3], A. ARKHIPOV[1], M. HAWRYLYCZ[1];
[1]Modeling, Analysis, and Theory, Allen Inst. For Brain Sci., Seattle, WA; [2]Human Cell Types, [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Identification of neuronal cell-types is one of the most fundamental step toward understanding and modeling of neuronal activity. As a part of our cell-type identification effort at the Allen Institute for Brain Science, we present a fully data-driven approach to a robust electro-physiological (ephys) type neuronal identification in the mouse primary visual cortex (V1) using transcriptomics, morphology, and ephys data.
1111 neurons were collected from V1 over all layers using 12 Cre-lines. The recordings are made with various stimuli to characterize their ephys properties. Overall 311 features including single action potential features, action potential train features, and cell level ephys features were calculated. Clustering trees were built by iterative PCA or by Gaussian mixture model. Robust cluster assignment was made by co-clustering analysis over multiple clustering runs using a 10 fold cross validation resampling strategy. Applying these methods to an initial data set collected from mouse V1 resulted in 10 cluster clustering tree. The initial branch separated inhibitory and excitatory neurons. Subsequent branches further distinguished the clusters into 3 fast spiking, 2 moderate width spiking, and 5 regular spiking groups. These groups were also assessed by comparing intrinsic ephys properties, cortical layer, and Cre-line composition to other cell-type classifications of cortical neurons previously reported in the literature. These comparisons with earlier studies revealed areas for further investigation and refinement. Keywords : Cell-type, Ephys-type, Clustering, Co-clustering, iterative PCA, Gaussian mixture model

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Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: RGPIN-2015-06215

Title: A modern approach to labelling and visualizing the central visual pathway

Authors: ***J. L. BALSOR**, K. M. MURPHY;
McMaster Integrative Neurosci. Discovery and Study, McMaster Univ., Hamilton, ON, Canada

Abstract: Recent tissue clearing protocols provide exciting opportunities for studying connections from the eye to the brain. There is a problem, however, combining pathway tracing with the see-through brain because traditional tracers (e.g. proline, WGA-HRP) are not compatible with tissue clearing. Here we describe a simple and reliable solution that takes advantage of a new tracer using wheat germ agglutinin labelled with a fluorescent dye (CF dyes, Biotium Inc.). WGA is actively taken up by retinal ganglion cells, anterogradely transported to LGN and superior colliculus, and moves trans-synaptically to label inputs to visual cortex. The fluorescent dye is water soluble, highly photostable, bright and comes in a rainbow of 20 colours from blue (350nm) to far-red (770nm).Our protocol combines eye injections with CF dye-labelled WGA and PAssive Clarity Technique (PACT) to visualize the central visual pathway. We did an injection of 5% CF dye-labelled WGA (CF770R or CF680R) into the posterior chamber of each eye. The timeline was: injections on days 1 and 3, then perfusion with PBS on day 5. The retinae were removed and imaged to verify labelling of retinal ganglion cells. Both cortical hemispheres were resected, then unfolded and flattened by removing white matter and making a medial and lateral cut to relieve the intrinsic curvature. The cortex was gently flattened between glass slides and fixed in 2% PFA for 30 minutes. A dissection was done to remove the intact subcortical visual pathway, including optic nerves, optic radiations, LGNs and superior colliculi. This was post-fixed in 2% PFA at 4°C for 5 hours. The PACT procedure was used to clear the tissue. Briefly, tissue was placed in a hydrogel monomer solution (4% acrylamide) and incubated at 4°C for 2 days, then polymerized at 37°C for 5 hours and cleared with a mild detergent (8% SDS) at 37°C until tissue was transparent (range 4-14 days). We did regular low magnification imaging using LiCor Odyssey NIR Scanner to see labelling before, during and after clearing. We found bright labelling of the visual pathway at all stages, including before

tissue clearing. As the tissue cleared more details of the pathway became apparent. These features include layers in LGN, and patches in colliculus and visual cortex. We also did high magnification imaging using confocal microscopy and found lots of vesicularly packaged label in axons, dense clumps of label at presumptive synapses but no label in blood vessels.This modern protocol is fast and easy, plus the label is compatible with cutting-edge techniques (super resolution), making it ideal for studies spanning questions from nano- to macroscales.

Disclosures: J.L. Balsor: None. **K.M. Murphy:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: NINDS Competitive Fellowship Award

NICHD Intramural Training Award

Title: Interneurons derived from the caudal ganglionic eminence are preferentially connected to callosal projecting pyramidal cells in deep cortical layers

Authors: *J. C. WESTER[1], C. J. MCBAIN[2];
[2]NICHD, [1]NIH, Bethesda, MD

Abstract: Inhibitory interneurons are crucial regulators of cortical activity, acting to gate the flow of excitatory activity and regulate network oscillations. Although highly diverse, cortical interneurons can be segregated into two non-overlapping subgroups based on their embryonic lineage from either the caudal or medial ganglionic eminences (CGE and MGE). Similarly, cortical excitatory principal cells (PCs) can be segregated based on the target of their long-range axonal projection: those projecting to cortex/striatum (intratelencephalic ICPNs) and those projecting to the brainstem, thalamus, and spinal cord (subcerebral SCPNs). ICPNs and SCPNs form local parallel overlapping microcircuits and recent evidence indicates that they may be differently regulated by CGE or MGE interneurons. Here, we tested the hypothesis that CGE-derived interneurons and ICPNs form preferential synaptic connections. We used dual whole-cell patch clamp recordings to test for synaptic connections in layer 5 of mouse visual cortex, where ICPNs, SCPNs, and CGE- and MGE-derived interneurons are intermingled. We used the *Htr3a*-GFP mouse line to target CGE-derived interneurons, and retrobead injections into the contralateral visual cortex or ipsilateral superior colliculus to target ICPNs or SCPNs,

respectively. We found that CGE-derived interneurons indeed formed synaptic connections onto ICPNs with greater probability than SCPNs, but in both cases was low (7% vs. 3% connectivity). Interestingly, the most striking difference was the connectivity from PCs to interneurons. Specifically, the probability of finding an excitatory synaptic connection from an ICPN to a CGE-derived interneuron was 11%, compared to 0% for SCPNs. In order to confirm the apparent absence of excitatory input from SCPNs, we injected g-deleted rabies encoding channelrhodopsin (ChR2) and the fluorophore mCherry into superior colliculus. Optical excitation of a local population of SCPNs resulted in only 4 of 14 CGE-derived interneurons exhibiting monosynaptic excitatory input, which was on average less than 10 pA in amplitude when observed. Thus, SCPN input onto CGE-derived interneurons exists but is very sparse and weak. Finally we used paired whole-cell recordings to test synaptic connectivity in layer 2/3, where all PCs are ICPNs and CGE interneurons are in the majority compared to those from the MGE. We found that CGE interneurons innervated PCs with a probability of 12%, and PCs innervated CGE interneurons with a probability of 23%. Thus, excitatory drive from ICPNs to CGE-derived interneurons is prevalent throughout cortical layers and likely represents an important canonical circuit motif.

Disclosures: **J.C. Wester:** None. **C.J. McBain:** None.

Poster

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Title: Learning changes the selectivity and interactions of GABAergic interneuron classes in visual cortex

Authors: *J. POORT[1], A. G. KHAN[2], A. BLOT[2], S. B. HOFER[2], T. D. MRSIC-FLOGEL[2]; [1]Univ. Col. London, London, United Kingdom; [2]Biozentrum, Basel, Switzerland

Abstract: Neural representations of sensory stimuli can be modified by experience. In the mouse primary visual cortex (V1), responses become increasingly selective when animals learn the behavioural relevance of visual stimuli. However, it is unclear how learning reorganises the activity of the different cell types, including excitatory pyramidal (PYR) neurons and different classes of GABAergic interneurons. Although pyramidal cells provide the output from the local circuit to other cortical areas, different interneuron classes can inhibit pyramidal cells as well as each other, and thus exert a powerful influence on circuit activity. To understand how learning changes the responses and co-activation patterns of the different cell types, it is necessary to measure their activity at the same time.

We used two-photon calcium imaging of GCaMP6f signals to record responses of neuronal populations in layer 2/3 of V1 as mice learned to discriminate two visual patterns while running through a virtual corridor, by selectively licking in response to only one of the patterns to obtain a reward. After the behavioural experiments we co-registered immunostained brain sections with in-vivo recording sites to identify simultaneously imaged parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP) interneurons.

Within and across cell classes, neurons exhibited a large degree of heterogeneity in their responses to the task-relevant stimuli, as well as how the amplitude of their responses changed with learning. Response selectivity for task relevant stimuli increased preferentially in PYR and PV cells. Interestingly, PV cells became as selective as PYR cells after learning. Moreover, response correlations decreased over learning not only between cells belonging to the same class, but more strikingly, between cells from different classes. SOM cells in particular exhibited a marked reduction in correlations with all other cell classes, and a subset developed negatively correlated activity fluctuations with VIP and PV cells.

To determine whether these changes were specific to learning, we trained the same mice to switch between attending to or ignoring the visual stimuli. Although we observed comparable changes in selectivity, we found that learning and attentional switching modulated responses in different sets of PYR cells, and that attentional switching, in contrast to learning, was not associated with strong reductions in correlated activity. These results demonstrate how learning the behavioural relevance of visual stimuli leads to specific and concerted changes in the selectivity and co-activation patterns across multiple cell classes.

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Poster

433. Rodent Visual Cortex

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Support: ERC Starting grant 281885 - PERCEPT

DFG EXC 307

Title: How does behavioral relevance affect neural responses in mouse primary visual cortex and thalamus?

Authors: *A. WAL[1,2], A. VAICELIUNAITE[1], P. GEORGIEVA[1], L. BUSSE[1,3], S. KATZNER[1]; [1]Ctr. For Integrative Neurosci., Univ. of Tuebingen, Tuebingen, Germany; [2]Grad. Sch. of Neural and Behavioural Sci., Intl. Max Planck Res. Sch., Tuebingen, Germany; [3]Div. of Neurobiology, Dept. Biol. II, LMU Munich, Munich, Germany

Abstract: In the mouse early visual system, neural responses can reflect the behavioral relevance of a visual stimulus (Poort et al., 2015; Wimmer et al., 2015). Current work aims at understanding the circuit-level mechanisms, by which behavioral relevance can shape sensory processing. Here, we ask how behavioral relevance affects responses of inhibitory interneurons in primary visual cortex (V1). In addition, we ask whether it can influence sensory responses already at the level of the dorsolateral geniculate nucleus (dLGN). We designed a visual foraging paradigm, in which two stimuli provide identical drive to V1 neurons, but differ in reward contingencies. Head-fixed mice were placed on a spherical treadmill, where they could start a trial by moving forward. The stimuli consisted of a single drifting grating, which could appear either behind a square or diamond aperture. For one stimulus, mice could earn a fluid reward by running for an additional 4 s; the other stimulus had no consequences. At any point in time, mice could reject a stimulus by slowing down and thereby terminate the current trial. We recorded extracellular activity with multi-contact silicon probes spanning the depth of V1 or dLGN. In a passive viewing condition, we measured sensory responses to the two stimuli outside the context of the task. We used these measurements to exclude from further analyses all neurons that showed any difference in their sensory response to the two stimuli. We recorded, during task performance, from ensembles of V1 neurons and used optogenetic tagging to identify inhibitory interneurons expressing parvalbumin (PV+). We compared responses to the two stimuli within a time window of 500 ms following stimulus onset, and identified two classes of neurons: some increased the stimulus-driven response if the animal could earn a reward (‘positive modulation’), others if there was no prospect of reward (‘negative modulation’). Identified PV+ neurons tended to show negative modulation. Responses of putative pyramidal cells, on the other hand, seemed more balanced, showing positive as well as negative modulation. These cells were spread across

the depth of cortex, including the thalamo-recipient layer 4. Consistent with such a laminar pattern, we found that task performance already modulated responses of dLGN neurons. Our findings show that the visual foraging paradigm can be used to manipulate behavioral relevance while keeping the sensory input for single neurons constant. These manipulations can affect sensory processing within the mouse early visual system, potentially including inhibition by PV+ interneurons and thalamic responses.

Disclosures: **A. Wal:** None. **A. Vaiceliunaite:** None. **P. Georgieva:** None. **L. Busse:** None. **S. Katzner:** None.

Poster

433. Rodent Visual Cortex

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 433.21/SS5

Topic: D.06. Vision

Title: Generation of biophysically-detailed models that reflect diverse intrinsic properties of cortical neuron types

Authors: ***N. W. GOUWENS**, J. BERG, T. DESTA, D. FENG, T. FLISS, K. GODFREY, T. JARSKY, C. LEE, S. SORENSEN, S. SUNKIN, Z. ZHOU, A. BERNARD, C. DANG, L. NG, H. PENG, J. PHILLIPS, H. ZENG, M. HAWRYLYCZ, C. KOCH, A. ARKHIPOV;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Cortical neurons exhibit a variety of intrinsic electrophysiological properties that can shape their roles in the cortical circuit. Network models built to investigate these effects on cortical processing require models of individual neurons that reflect this diversity across cell types. To address this need, we have used the experimental data contained in the Allen Cell Types Database to construct biophysically detailed models that reproduce the characteristics of action potential firing of individual neurons. These models are based on whole-cell electrophysiological recordings from mouse visual cortical slices, as well as the 3D morphologies reconstructed from biocytin fills of the same cells. The majority of the recorded cells were labeled by a variety of Cre driver lines, including ones that exhibit enriched expression in specific cortical layers. We fit the models using the NEURON simulation environment by placing at the soma a set of ~10 mechanisms describing active conductances and intracellular calcium dynamics, then adjusting the densities of these mechanisms via a genetic algorithm until 12 firing property features (e.g., action potential width, action potential peak, average firing rate) matched the original experimental data. We identified a standardized set of techniques that produced well-fit models reliably across our data set, which encompassed a

variety of firing patterns and dendritic morphologies. We also automated these techniques with open-source software packages, including the Allen Software Development Kit (AllenSDK). Models fit by these methods generalized well to a variety of stimuli, such as noisy current injection and current ramps, which we assessed by comparing the model to responses from the recorded cells evoked by identical stimuli. In addition, we used unsupervised clustering methods on the broader Allen Cell Types Database to identify data-driven classes based on electrophysiological properties. We found that the models could be reliably assigned by a supervised learning method to the classes of the original cells, suggesting that our model set preserves diversity in intrinsic properties found experimentally. Finally, we have made these 150+ models publically available online, in addition to providing the source code to run them as part of the AllenSDK.

Disclosures: **N.W. Gouwens:** None. **J. Berg:** None. **T. Desta:** None. **D. Feng:** None. **T. Fliss:** None. **K. Godfrey:** None. **T. Jarsky:** None. **C. Lee:** None. **S. Sorensen:** None. **S. Sunkin:** None. **Z. Zhou:** None. **A. Bernard:** None. **C. Dang:** None. **L. Ng:** None. **H. Peng:** None. **J. Phillips:** None. **H. Zeng:** None. **M. Hawrylycz:** None. **C. Koch:** None. **A. Arkhipov:** None.

Poster

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Title: Circuit models with multiple types of inhibitory neurons based on a brain-wide map of cell density

Authors: ***G.-Y. R. YANG**[1], L. C. GARCIA DEL MOLINO[1], Y. KIM[2,3], P. OSTEN[2], X.-J. WANG[1,4];
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Harbor, NY; [3]Penn State Univ., Hershey, PA; [4]NYU-ECNU Joint Inst. of Brain and Cognitive Sci., Shanghai, China

Abstract: The brain consists of various types of interneurons that differ in their morphology, physiology, and connectivity. In the mammalian cortex, 80% of all interneurons are accounted by three major non-overlapping cell types: neurons expressing parvalbumin (PV), somatostatin (SST), or vasoactive intestinal peptide (VIP). The physiological properties of single interneurons are relatively well known at this point. These interneurons form a canonical circuit motif that was recently mapped in several areas. Yet the dynamics of such an interneuronal circuit is-as we show-far from being well understood.

In particular, the circuit dynamics depend critically on the cell density of each type of interneuron. For one type of neuron, having a higher density can lead to stronger output connections on the population level. We quantitatively measured the density of PV-, SST-, and VIP-expressing interneurons across the whole mouse brain (Kim et al. SFN 2015). Our data show that the density of each type of interneuron varies greatly across cortical areas, with the PV neuron density varying more than five-fold, and SST-neuron density more than three-fold.

We investigated the functional implications of this density variation in both simple population-based models and biological-realistic spiking neural circuit models. Through numerical simulation and mathematical analysis, we found that varying the density of interneurons can have counterintuitive impacts on the circuit dynamics. Specifically, we studied the effective connectivity and synaptic current from one class of neuron to another. One paradoxical effect we uncovered, for example, was that when external inputs target PV neurons, elevating the density of SST neurons will decrease the effective connectivity yet increase the synaptic current from PV to excitatory neurons.

Connecting these local circuits through quantitative long-range connections, we built a large-scale dynamical circuit of the mouse cortex. We report that the uneven interneuron density across cortex greatly alters the functional connectivity between areas, including stronger functional couplings within frontal areas. This finding suggests that models of large-scale cortical circuits need to take into account the heterogeneity of interneurons across areas.

Disclosures: G.R. Yang: None. **L.C. Garcia del Molino:** None. **Y. Kim:** None. **P. Osten:** None. **X. Wang:** None.

Poster

433. Rodent Visual Cortex

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Topic: D.06. Vision

Support: R01MH062349

Title: Cortical microcircuit endowed with three interneuron subtypes exhibits a repertoire of multiple dynamical regimes.

Authors: *L. GARCIA DEL MOLINO, G. R. YANG, X.-J. WANG;
New York Univ., New York, NY

Abstract: Recent anatomical studies in mice have revealed a recurrent pattern in the connectivity among excitatory pyramidal neurons and three major subpopulations of inhibitory neurons expressing parvalbumin, somatostatin or vasoactive intestinal peptide, respectively. Dissecting the dynamics of this canonical microcircuit is essential to our understanding of the mammalian cortex.
To this goal, we built a computational model that reproduces accurately the single-neuron behavior of each class of neurons based on physiological data and used it to study the impact of different connection weights on the collective circuit dynamics. We explored systematically the connectivity space and analyzed the network behavior for both spontaneous and input driven activity. We find that, in comparison with classical networks composed of one excitatory and one inhibitory population, networks with several classes of interneurons have a much richer repertoire of dynamical behaviors, some of them highly counterintuitive. For example, under certain condition
We conducted a systematic mathematical analysis of a simpler neuronal model that predicts and explains our observations, showing that they are reproducible and robust.
This new network paradigm opens a new playground for studying recurrent neuronal network dynamics. Our work also provides suggestions and predictions for future experiments on systems that include multiple interneuron populations.s for the circuit connections, the activity of the excitatory population may decrease with an increase in its external input.
We conducted a systematic mathematical analysis of a simpler neuronal model that predicts and explains our observations, showing that they are reproducible and robust.
This new network paradigm opens a new playground for studying recurrent neuronal network dynamics. Our work also provides suggestions and predictions for future experiments on systems that include multiple interneuron populations.

Disclosures: L. Garcia Del Molino: None. **G.R. Yang:** None. **X. Wang:** None.

Poster

433. Rodent Visual Cortex

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Simons Collaboration on the Global Brain (325295)

RPB Stein Innovator Award

Title: Stimulus-specific response enhancement in mouse primary visual cortex depends on locomotion

Authors: *M. KANEKO[1], Y. FU[2], M. P. STRYKER[1];
[1]Physiol. and Ctr. for Integrative Neurosci., Univ. of California, San Francisco, CA; [2]Singapore Bioimaging Consortium, Agency for Sci. Technol. and Res., Singapore, Singapore

Abstract: Many experiments have found that responses in adult mammalian visual cortex are stable over long periods. Direction selectivity is the only property widely reported to be malleable (Wu *et al.*, *J Neurosci*, 2011), but evoked potential recordings through implanted electrodes in adult mice are reported to reveal a rapid and persistent increase in response to repeated stimuli (Frenkel *et al*., Neuron, 2006; Cooke & Bear, *J Neurosci*, 2010). Here, we used intrinsic signal imaging through the intact skull, a completely non-invasive technique, to investigate whether repeated exposure to specific stimuli would enhance visual responses in adult mouse primary visual cortex (V1). After acclimating to the setting, wild type adult (postnatal day 70 - 90) C57B6/J mice were allowed to run on Styrofoam balls floating on air while viewing one of three different, high-contrast visual stimuli 2 hours per day for 5 days, as described previously (Kaneko and Stryker, *eLife*, 2014). We found that V1 responses to the stimuli that were viewed by the animal were specifically enhanced, leaving responses to other stimuli unaffected. The enhancement was prevented by an NMDA receptor antagonist and persisted for at least a week following cessation of 10 days of stimulus exposure. Similar exposure in mice that were not walking or running did not significantly enhance responses. When animals were given little opportunity for locomotion, the response enhancement was observed in mice walking for only 40 - 60 minutes over 10 days.

To start to understand cellular mechanisms underlying this enhancement, we followed responses of single neurons in layer 2/3 before and after the daily exposure to one of 8 different directions of drifting gratings with 2-photon calcium imaging using virus-mediated GCaMP6s expression. In majority of cells, the response magnitude to the exposed orientation was significantly increased, while that to the orthogonal orientation was unchanged. Moreover, the preferred orientation in most cells that were originally selective for orientations near the one to which they were exposed shifted slightly but significantly toward the exposed stimulus. In control animals that experienced locomotion without exposure to high-contrast visual stimuli, orientation tuning was stable in nearly all cells.

These findings indicate that stimulus-specific plasticity in the adult visual cortex depends on concurrent locomotion, presumably as a result of the high-gain state of visual cortex induced by locomotion (Fu *et al*., *Neuron*, 2014).

Disclosures: **M. Kaneko:** None. **Y. Fu:** None. **M.P. Stryker:** None.

Poster

434. Striate Cortex Plasticity I

Location: Halls B-H

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Topic: D.06. Vision

Support: NIH Grant R01 AA22455

Title: Role of CREB, SRF and MEF2 in ocular dominance plasticity

Authors: ***N. S. PULIMOOD**, A. E. MEDINA;
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Abstract: The transcription factors CREB (cAMP Response Element Binding factor), SRF (Serum Response Factor) and MEF2 (Myocyte Enhancer Factor 2) play critical roles in neuronal plasticity, and have been shown to differentially affect underlying plasticity mechanisms like LTD and LTP. Additionally, a recent study showed that CREB, SRF and MEF2 control the expression of the early gene Arc, which is required for the expression of ocular dominance plasticity (ODP), by binding to the synaptic activity responsive element (SARE). However the role of the activation of these transcription factors in the different components of plasticity *in vivo* is not well known. We used Visually Evoked Potentials (VEPs) in awake mice to investigate the role of CREB, SRF and MEF2 on the depression and potentiation components of ODP (dc-ODP and pc-ODP). We infected animals with a Herpes Simplex viral (HSV) vector expressing dominant negative forms of CREB (CREB-DN), SRF (SRF-DN) or MEF2 (MEF2-DN), then chronically implanted electrodes in the binocular zone of the mouse visual cortex. We then recorded VEPs before and after a period of monocular deprivation (MD). Since the two components of ODP express in a temporally distinct manner in the mouse visual cortex, we used 3 days of MD to isolate dc-ODP and 7 days of MD to investigate pc-ODP. Our results show that these three transcription factors have different effects on dc-ODP and pc-ODP; CREB and MEF2 seem to block both dc-ODP and pc-ODP, whereas SRF seems to block only dc-ODP. Our results can elucidate the role of these key players in the two components of ODP, therefore better informing future attempts to create therapeutic targets for diseases such as amblyopia where ODP is defective.

Disclosures: **N.S. Pulimood:** None. **A.E. Medina:** None.

Poster

434. Striate Cortex Plasticity I

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: D.06. Vision

Support: Natural Sciences and Engineering Research Council of Canada

Title: Orientation tuning curves and selectivity in V1: influence of the past stimulus

Authors: *F. ETINDELE SOSSO, V. BHARMAURIA, L. BACHATENE, S. CATTAN, A. OUELHAZI, N. CHANAURIA, S. MOLOTCHNIKOFF;
Biol. Sci., Univ. of Montreal, Montreal, QC, Canada

Abstract: The knowledge of past influences the way we experience the world. That is, any visual stimulus although extinguished leaves a trace that affects responses of the next stimulus. It has been demonstrated that neuronal responses of visual neurons are largely dependent upon the visual background and the stimuli properties that precede the actual tested light visual stimulus. Many investigators have explored the effect of adaptation (more than 6 min) on the orientation tuning of neurons, but to our knowledge, there is no documentation of how the orientation selectivity of visual neurons is influenced to the application of an initial short (ms level)random or short fixed stimulus before the test (experiencing) stimulus. To this goal, in the primary visual cortex of urethane anaesthetized mice, using orientation tuning curve as a model, we investigated the impact of a briefly applied (250 ms duration, history stimulus) oriented grating (BAOG) on the responses evoked by the succeeding oriented tested grating lasting 4 s (OTG). Two basics protocols were employed and compared: in the first, the BAOG had a fixed angle (that is, it remained unchanged). In the second, the BAOG randomly varied prior to the experiencing grating. The stimuli properties were same in either case. In both cases, the orientation tuning curves were investigated prior to- and post-BAOG. The results demonstrated that the fixed BAOG (analogous to adaptation) significantly shifted the peak of the tuning curve (mean shift = 35.5°, t-test, $p < 0.0001$), increased the OSI (Δ OSI = 0.09, t test $p < 0$, 0001) and decreased the bandwidth (Δ bandwidth =1.4, t-test $p < 0.0001$) of neurons. On the contrary, the random BAOG significantly lead to a decreased OSI (Δ OSI = 0.05, t test $p < 0.0001$), increased responses in the flanked orientation (Δ FR = 18.98, t test $p < 0.0001$) without a change in optimal responses (Δ FR = 0.048), and increased bandwidth (Δ bandwidth = 1.0°, t-test $p < 0.0001$). These results suggest that the previous stimuli differentially affect the dynamics of neuronal responses and their selectivity. ***This research is supported by Natural Sciences and Engineering Research Council of Canada***

Disclosures: F. Etindele Sosso: None. **V. Bharmauria:** None. **L. Bachatene:** None. **S. Cattan:** None. **A. Ouelhazi:** None. **N. Chanauria:** None. **S. Molotchnikoff:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: NIH grant DP2EY024504-01

Title: The role of Neuregulin1/ErbB4 signaling in transplant-induced cortical plasticity

Authors: *X. ZHENG[1], T. IKRAR[2], X. XU[2], S. P. GANDHI[3];
[2]Dept. of Anat. and Neurobio., [3]Dept. of Neurobio. and Behavior, [1]Univ. of California, Irvine, Irvine, CA

Abstract: In postnatal development, the maturation of GABAergic interneurons promotes the experience-dependent refinement of cortical circuits. Previously, we have shown that transplantation of embryonic GABAergic interneurons reactivates a new critical period for ocular dominance plasticity in adult visual cortex. Here, we investigate the cellular and molecular mechanisms that mediate interneuron transplant-induced plasticity. Neurotrophic factor neuregulin 1 (NRG1) and its receptor ErbB4 have been shown to play a key role in the developmental strengthening of excitatory synapses onto inhibitory neurons, as well as in maturation of the inhibitory synaptic transmission. In this study, we investigated the role of neuregulin1/ErbB4 signaling in transplanted-induced ocular dominance plasticity. First, we transplanted embryonic GABAergic interneurons into adult primary visual cortex. Thirty-five days after transplantation, the animals underwent 4 days of monocular deprivation while receiving either daily injections of soluble NRG1 or saline control. Intrinsic signal optical imaging revealed that NRG1 blocked the reactivation of ocular dominance plasticity by transplanted interneurons. The synaptic locus of the blocked reactivation of plasticity is being investigated using *in vivo* 2-photon Ca^{2+} imaging of visual responses and *in vitro* circuit mapping. Altogether, these results are consistent with the hypothesis that excess NRG1 prevents the reopening of critical period for ocular dominance plasticity by accelerating the maturation of transplanted inhibitory neurons.

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Poster

434. Striate Cortex Plasticity I

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Support: Canadian Institute for Health Research MOP-111003

Natural Sciences and Engineering Research Council of Canada 238835-2011

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Title: Cholinergic enhancement accelerates recovery of vision after optic nerve damage.

Authors: *M. CHAMOUN[1], E. SERGEEVA[2], P. HENRICH-NOACK[2], S. JIA[2], L. GRIGARTZIK[2], J. MA[2], Q. YOU[2], F. HUPPÉ-GOURGUES[1], B. A. SABEL[2], E. VAUCHER[1]; [1]Optometrie, Univ. De Montreal, Montreal, QC, Canada; [2]Inst. of Med. Psychology, Otto-von-Guericke Univ., Magdeburg, Germany

Abstract: Enhancing cortical plasticity and brain connectivity following a visual impairment may improve residual vision. It is possible that these plasticity changes could be achieved by perceptual training or activation of the cholinergic transmission, acetylcholine being a neuromodulator involved in attention and neuronal plasticity. Here we study whether the cholinergic activation improves vision restoration by examining the effect of the acetylcholinesterase inhibitor donepezil on behavioral, electrophysiological and morphological parameters of visual function after the partial optic nerve crush in adult rats. Residual vision recovery was measured by following the rat's performance in a brightness discrimination task for 4 weeks. Neuronal activity was evaluated by quantification of visually evoked potentials once a week for the 4 weeks post-lesion. Visual cortex reactivity was evaluated by thallium autometallography post mortem. Cholinergic enhancement by donepezil induces faster recovery of brightness discrimination performance in rats with optic nerve crush compared to controls. However, visually evoked neuronal activity was not restored. This finding is compatible with the view that restoration of visual function may involve mechanisms beyond the area of primary damage and opens a new perspective for improving visual rehabilitation in humans.

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Poster

434. Striate Cortex Plasticity I

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Support: FRQS Postdoctoral Fellowship

FWO Postdoctoral Fellowship

FWO Research Project

Title: Brain-wide analysis of parvalbumin, somatostatin and vasointestinal peptide levels in cortical interneurons of sighted and enucleated mice using a newly developed imaging tool

Authors: *M.-E. LARAMEE, S. VREYSEN, L. ARCKENS;
KU Leuven, Leuven, Belgium

Abstract: ***Introduction:*** Monocular enucleation (ME) in mice causes a decrease of activity in the contralateral monocular visual cortex, which is gradually reactivated over the course of 7 weeks (Van Brussel et al. 2011; Nys et al. 2014). Parvalbumin interneurons were shown to be key regulators of the reactivation (Nys et al. 2015). In this study, we aimed at identifying the relation between excitation and inhibition using *in situ* hybridization (ISH) for the activity marker *zif268* and for markers of the three main inhibitory neuron populations: parvalbumin (PV), somatostatin (SOM) and vasointestinal peptide (VIP) in ME mice. ***Method:*** To provide a complete overview of the brain-wide expression with high spatial resolution, we built a software tool which allows us to register series of ISH coronal slices from adult C57BL/6J mice that were either sighted controls or enucleated. This tool allows mapping areal borders, segmenting the cortex and calculating the optical densities of the signal within these segments. The segmented cortex is reconstructed in 3D and projected to a horizontal plane creating a top view of the brain. Quantification of the differences between the top view images between ME and control mice is performed using a pseudo t-test to match the need for a non-parametric testing approach. ***Results:*** The developed tool generated high-resolution top view images comparable with optical imaging images. The expression patterns of the activity marker *zif268*, and of the inhibitory markers *pv*, *som* and *vip*, were visualized over the entire mouse visual cortex before and after ME. Complementary expression patterns were found between the different markers in sighted control mice. Surprisingly however no effect of ME was found on the expression levels of the three inhibitory markers, whereas the *zif268* expression was strongly affected, consistent with our previous results (Van Brussel et al. 2011; Nys et al. 2014). ***Conclusion:*** The tool designed is versatile and powerful as it allows combining information from different stainings from the same animal or condition to facilitate the interpretation of correlations between different molecular

patterns across the cortex with high spatial resolution. In our present study, it allowed us to describe the complementary patters of the different markers and to statistically compare expression patterns between different experimental groups. The lack of changes in expression of the inhibitory markers in our ME mice could indicate that changes occur at the protein level rather than at the mRNA level.

Disclosures: **M. Laramee:** None. **S. Vreysen:** None. **L. Arckens:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: NIH Grant EY10217

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Research to Prevent Blindness

Title: Cortical representation of a myopic peripapillary crescent: evidence against fill-in of retinal lesions

Authors: ***D. L. ADAMS**, J. R. ECONOMIDES, J. C. HORTON;
Ophthalmology, UCSF, San Francisco, CA

Abstract: Subjects who become myopic often develop a crescent-shaped region of retinal damage around the temporal border of the optic disc, due to axial stretching of the globe. This region is known as a myopic peripapillary crescent. There are no photoreceptors in this atrophic zone, and partial or complete absence of other retinal layers. Consequently, the blind spot becomes enlarged. It is unknown whether enlargement of the blind spot, a process that occurs after the critical period for plasticity of ocular dominance columns, can be detected in the primary visual cortex. We examined the cortical representation of the blind spot in three macaque monkeys using cytochrome oxidase (CO) histochemistry and/or [^{3}H]proline autoradiography. Two animals were normal, but the third had acquired peripapillary atrophy from high myopia. At age 2 years its refraction was +0.50 sphere OU and the optic discs appeared normal. At age 8, the refraction was -12.50 sphere in the right eye, -13.75 sphere in the left eye. The optic discs were tilted, and bordered temporally by a zone of peripapillary atrophy nearly half the width of the optic disc itself. In the visual cortex there was a zone where CO histochemistry revealed a few pairs of alternating dark and light ocular dominance columns. The

dark and light columns were equal in width. The density of the dark columns matched the background level of cortical CO activity. The ocular dominance columns were situated in the retinotopic map at precisely the location in striate cortex where the temporal side of the optic disc is represented. The most likely inference is that peripapillary damage from high myopia caused loss of retinal drive, resulting in down regulation of metabolic activity within ocular dominance columns serving the contralateral eye. The cortical defect matched the myopic peripapillary crescent in size and shape, indicating that "fill-in" of the retinotopic map by visual input from healthy surrounding retina does not occur, even for a lesion only a few degrees wide.

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Poster

434. Striate Cortex Plasticity I

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ERA-NET Neuron Grant, Neuro-DREAM

Title: Short-term deprivation of the amblyopic eye, combined with physical exercise, promotes long-term visual recovery in adult anisometropic patients.

Authors: *C. LUNGHI[1], A. SALE[4], A. LEPRI[2], A. SFRAMELI[3], A. DENDRAMIS[2], D. LISI[2], M. LEPRI[2], M. C. MORRONE[1];
[1]Traslational Res. and New Technologies on Med. and Surgery, [2]Ophthalmology Unit, Dept. of Surgical, Medical, Mol. and Critical Area Pathology, Univ. of Pisa, Pisa, Italy; [3]Ophthalmology Unit, Dept. of Surgical, Medical, Mol. and Critical Area Pathology, Univ. of Pisa, PISA, Italy; [4]Neurosci. Inst., Natl. Res. Council, Pisa, Italy

Abstract: We have recently shown that the adult visual cortex retains a high degree of neuroplasticity: short-term monocular deprivation (2h) unexpectedly boosts the deprived eye signal during binocular rivalry (Lunghi et al 2011), and decreases intracortical GABAergic inhibition (Lunghi et al, 2015). Interestingly, the effect of short-term monocular deprivation is further enhanced by moderate levels of physical activity (Lunghi & Sale, 2015). As boosting brain plasticity is fundamental for the treatment of amblyopia, especially in adulthood, we attempted a counterintuitive experiment where we deprived the amblyopic instead of the dominant eye, to promote the recovery of visual function in adult amblyopic patients, combining

monocular patching and physical activity. In five adult anisometropic patients (mean age 29±4 years, mean amblyopic eye acuity 0.32±0.24 LogMar), with no associated strabismus, who had not had treatment in the last 10 years, we patched the amblyopic eye for two hours over three consecutive days, then once per week over the next three weeks. During the patching period patients watched a movie while intermittently cycling on an exercise bike. Before and after each patching session we measured binocular rivalry (orthogonal gratings, size 2°, SF: 2 cpd with different contrast for the two eyes to achieve balanced dominance), visual acuity (ETDRS charts), stereoacuity (TNO test) and letter contrast-sensitivity. Surprisingly, we found that the perceptual dominance of the patched eye increased after deprivation similar to the effect found in normal subjects. During the four weeks of testing, visual acuity and contrast sensitivity improved in all four patients (average visual acuity improvement: 0.18 LogMar), and 3/5 patients also recovered stereopsis (two of these patients were stereoblind before the procedure). Strikingly, the improvement in both visual acuity and stereopsis was preserved for at least 1 month after testing. These results demonstrate that amblyopic eye vision can be improved by transiently depriving the weak rather than the strong eye (Zhou et al, 2013), probably by activating homeostatic plasticity. Physical exercise may be crucial for the recovery by potentiating the plastic potential of the visual cortex as observed in animal models (Baroncelli et al, 2012).

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Poster

434. Striate Cortex Plasticity I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 434.08/SS16

Topic: D.06. Vision

Support: CRSNG

Title: Intriguing! Auditory stimulus shifts orientation selectivity of visual neurons in cat V1

Authors: ***N. CHANAURIA**[1], V. BHARMAURIA[1], L. BACHATENE[1], S. CATTAN[1], F. A. ETINDELE-SOSSO[1], J. ROUAT[2], S. MOLOTCHNIKOFF[1];
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Abstract: Multisensory stimulation can have a substantial impact on the basic visual perception. Non-visual input such as auditory stimuli can affect visual functioning in a myriad of ways. Numerous studies have demonstrated these alluring cross-modal relationships. For example,

anatomical and electrophysiological approaches in non-human primates (*Ghazanfar & Schroeder, 2006; Driver & Noesselt, 2008*) have provided evidence that multisensory interactions can be observed at early stages of sensory processing (*Adeli et al. 2014*). This body of evidence suggests that projections from the auditory cortex reach deeper layers of the visual cortex and vice versa. Another study by *Muckli et al. 2013* highlights the existence and importance of non-geniculate input to V1 by associated areas such as auditory cortex. Moreover, an fMRI report by *Vetter et al. 2014* displayed through task-based approaches in blindfolded healthy adults that, by solely performing an audio task, a response in the visual cortex could be observed. Therefore, primary areas such as V1 and A1 showcase high multisensory interaction, predominantly a modulatory influence in response to a complementary stimulus. In a recent study, (*Ibrahim et al. 2016*) authors have shown that auditory stimulus sharpens the selectivity of visual neurons. In the present investigation, we further examined the effect of sound on the shifts of orientation selectivity of simultaneously recorded supra- and infragranular layer visual neurons by presenting an auditory stimuli for 12 minutes. The recordings were performed in area 17 of the visual cortex in anaesthetised cats using tungsten multichannel depth electrode. The auditory stimulus (3s 74 dB SPL) was broadband noise-like (*Fritz et al. 2003*) and consisted of temporally orthogonal rippled combinations (TORC's) with varying frequency components. The stimulus was presented continuously and uninterruptedly for 12 min and was delivered by a pair of external loudspeakers positioned perpendicularly to the fixation axis of the animal. Our data show that, after 12 min presentation of the auditory stimulus, a population of visual cortical neurons attain new orientation selectivities. In addition, few layer II-III and V neurons lose their selectivity and become untuned. Further, layer II-III and layer V neurons exhibit functional synchronisation (as computed by crosscorrelograms) prior to- and post- auditory stimulus exposure. These results suggest that, visual neurons in either layers change their properties on application of an auditory stimulus which highlights the cross-modal interactions between visual and auditory systems and a robust reconfiguration of visual cortex.

Disclosures: **N. Chanauria:** None. **V. Bharmauria:** None. **L. Bachatene:** None. **S. Cattan:** None. **F.A. Etindele-Sosso:** None. **J. Rouat:** None. **S. Molotchnikoff:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: European Research Council Grant ERC-2009-AdG 249425-CriticalBrainChanges

Title: Evidence for an intact retinotopic organization of early visual cortex but impaired extrastriate processing in sight recovery individuals

Authors: *S. SOURAV[1], D. BOTTARI[1], R. BALACHANDAR[2], R. KEKUNNAYA[2], B. RÖDER[1];
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Abstract: Congenital visual deprivation has been shown to cause extensive structural and functional changes in the brain. In periods when neural systems are particularly sensitive for experience (so called sensitive periods), visual deprivation can irrecoverably impair the ability to acquire visual functions if vision is subsequently restored. Some recent findings, however, have suggested that some neural systems emerge in the absence of developmental vision. Humans with a history of bilateral, dense congenital cataracts (CC) who subsequently underwent cataract-removal surgeries to restore vision provide a unique window into sensitive phases of visual development in humans. Using event-related potentials, we investigated the retinotopic organization of early visual cortex in CC individuals with a period of visual deprivation ranging from several months to several years.
Participants looked at grating stimuli flashed either in the upper or the lower visual field. The task of the participants was to detect rare gratings differing in orientation. Cataract individuals showed typical C1 waves of opposing polarities in response to visual stimulation from upper vs. lower visual fields, indicating that the retinotopic organization in the visual cortex is spared in the CC individuals. By contrast, the P1 wave, arising from extrastriate cortical sources, was attenuated in the CC group compared to their matched controls. The findings are in agreement with present brain imaging data suggesting intact retinotopy in congenitally permanently blind adults. We extend these results by demonstrating that the early visual cortex can be activated with the same latency after sight restoration as in healthy controls and thus, indeed is functional. Moreover, the findings are in accord with early suggestions of Hyvarinen et al. (1981) and permanently impaired visual function after visual restoration following a congenital blindness.
Reference Hyvarinen, J., et al. (1981). "Early visual deprivation alters modality of neuronal responses in area 19 of monkey cortex." Neurosci Lett 26(3): 239-243.
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Disclosures: S. Sourav: None. **D. Bottari:** None. **R. Balachandar:** None. **R. Kekunnaya:** None. **B. Röder:** None.

Poster

434. Striate Cortex Plasticity I

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AMED-CREST

KAKENHI-JSPS

MHLW-Japan

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HHMI

Title: Increasing Arc prolongs the critical period for juvenile plasticity in primary visual cortex

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Abstract: A defining feature of early postnatal brain development is the activity-dependent winnowing of synaptic connections. This process is readily demonstrated by the response of visual cortical circuits to temporary monocular deprivation (MD) during early life. When MD is initiated during an early sensitive period, the synapses serving the deprived eye in visual cortex lose strength and eventually are eliminated. Deprived-eye depression diminishes with age such that by the onset of adolescence, circuits are no longer vulnerable to deprivation. A question of extraordinary interest concerns the mechanisms that bring this critical period to a close. Clues into the molecular basis for the decline in juvenile plasticity have come from several diverse experimental treatments that can alter sensitivity to MD across the lifespan. It has been suggested that a common thread connecting these varied treatments might be an increase the ratio of excitation to inhibition. However, it is completely unknown how, at the molecular level, general increases in cortical activity increase deprivation-induced synaptic plasticity. We hypothesize that a key molecular determinant of juvenile synaptic plasticity—as demonstrated in visual cortex by depression of deprived-eye responses following MD—is expression of the activity-

regulated gene for Arc. Here we asked if activity-dependent overexpression of Arc prolongs into adulthood the sensitivity of visual cortical synapses to deprivation and if so, how. Using a novel genetically engineered mouse, in which an Arc transgene is expressed under the control of the activity-dependent Arc promoter, we show that augmenting Arc expression renders visual cortical synapses vulnerable to deprived-eye depression in adults. Further, we show in wildtype mice that Arc expression in visual cortex normally declines with age coincident with the loss of juvenile plasticity. Finally, we show that genetic deletion of Arc and chronic inhibition of protein synthesis in juvenile visual cortex, which eliminate ocular dominance plasticity, also disable the mechanism of homosynaptic long-term depression (LTD). The fact that Arc is activity-regulated provides an elegant explanation for how crude manipulations of the excitation-inhibition ratio can, under some circumstances, restore the sensitivity of visual cortex to MD.

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Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: HHMI

Gatsby Foundation

Title: Cortico-fugal output from visual cortex promotes plasticity of innate motor behavior

Authors: ***B. LIU**[1], A. HUBERMAN[1], M. SCANZIANI[2];
[1]UCSD, La Jolla, CA; [2]UCSD/HHMI, La Jolla, CA

Abstract: The cerebral cortex of mammals massively innervates the brainstem, a phylogenetically older structure, via cortico-fugal axonal projections. Many cortico-fugal projections target brainstem nuclei that mediate innate motor behaviors and yet, the function of these projections remains obscure. A prime example of such innate behaviors is the optokinetic reflex (OKR), a compensatory eye movement that stabilizes the image on the retina as the animal moves through the environment. This behavior is mediated by the brainstem nuclei of the accessory optic system (AOS) which receive direct visual input from the retina as well as visual cortex. The OKR is plastic throughout life as the organism matures and ages, and can be continuously readjusted relative to other oculo-motor reflexes. Although the cerebellum and

vestibular nuclei are known to be involved in the adaptive changes of the OKR, visual cortex could also contribute to this plasticity through cortico-fugal projections to the AOS. To experimentally induce OKR plasticity we performed vestibular lesions, a manipulation that leads to a persistent potentiation of the amplitude of the OKR. OKR potentiation was strongly reduced by optogenetically silencing visual cortex. Furthermore, targeted ablation of cortico-fugal neurons projecting to the AOS severely impaired OKR potentiation. Finally, we showed that OKR potentiation results from an enhanced drive exerted by visual cortex onto the AOS. Thus, cortico-fugal projections to brainstem enable visual cortex to plastically adapt the execution of innate motor behaviors. These findings expand our understanding of how the innervation of phylogenetically older structures by the mammalian cortex can improve the performance of reflexive behavior in an experience dependent manner.

Disclosures: **B. Liu:** None. **A. Huberman:** None. **M. Scanziani:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: Wellcome Trust Grant 095669

Wellcome Trust Grant 095668

Gatsby Foundation

Title: High-dimensional structure of inhibitory population activity in visual cortex

Authors: ***C. STRINGER**[1], M. PACHITARIU[2], M. DIPOPPA[2], M. OKUN[2], M. CARANDINI[2], K. HARRIS[2];
[1]Gatsby Computat. Neurosci. Unit, [2]Univ. Col. London, London, United Kingdom

Abstract: A fundamental feature of cortical circuits is the balance of excitation and inhibition. Intracellular recordings reveal that inhibitory synaptic inputs track fluctuations in excitatory inputs; similarly, in extracellular recordings, both putative inhibitory and excitatory neurons track the overall activity of the local network.
The activity of an excitatory neuron, however, is not fully summarized by a single population rate: for example, different neurons fire in response to different sensory stimuli or as part of different spontaneous ensembles. Describing the activity of an excitatory population thus requires a high-dimensional vector. For inhibitory neurons, it is unclear whether population

activity is high dimensional. Because inhibitory neurons receive dense, non-specific input from excitatory neurons, one might expect their firing to track the excitatory population rate, resulting in a one-dimensional space of inhibitory neuron activity.
To address this question, we analyzed population recordings made from mouse V1 with 2-photon calcium imaging, in which all neurons expressed the calcium indicator, and selected classes of inhibitory neurons (Pvalb, Sst, or Vip) were labelled with tdTomato. Contrary to expectation, we found that inhibitory population activity tracked excitatory activity along multiple dimensions. Using canonical correlation analysis, we found multiple subspaces of excitatory and inhibitory activity that were significantly aligned to each other. This property held for all three classes of inhibitory neurons. Preliminary evidence suggests that this property also holds for putative inhibitory neurons recorded electrophysiologically.
To provide insight into the possible mechanisms of this phenomenon, we analyzed multiple variants of a recurrent spiking network model. Model networks with purely random connectivity exhibited a single dominant mode of activity that included all excitatory and inhibitory cells. Adding structure only to the excitatory connectivity did not match our experimental data, as excitatory activity switched between full activation of discrete subnetworks, while inhibitory activity remained approximately constant. However, when we matched the inhibitory and excitatory connection structure the activity showed multiple inhibitory modes, matching our experimental findings.
We conclude that the high dimensional structure of inhibitory population activity may reflect organization of inhibitory synaptic networks. Our modelling work suggests that this structured inhibition permits stable high-dimensional computation.

Disclosures: C. Stringer: None. **M. Pachitariu:** None. **M. Dipoppa:** None. **M. Okun:** None. **M. Carandini:** None. **K. Harris:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: Howard Hughes Medical Institute

Gatsby Charitable Foundation

Title: Visual cortical activity during a virtual foraging task in mice

Authors: *A. RESULAJ[1], S. R. OLSEN[2], M. SCANZIANI[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Neural electrical activity in the brain represents the external world and guides our future actions. Although much is known about how sensory areas represent sensory information, little is known about how this information is used to guide behavior. Here, we recorded neuronal responses in primary visual cortex (V1) in mice while they performed a virtual foraging task. In this task, head-fixed mice ran on a circular treadmill while visual stimuli (circular patches of gratings rotated at either 45 or 90 degrees) shown on a monitor passed by at a speed that was proportional to their running speed. Mice were rewarded with water for stabilizing a specific image (the target stimulus) on the center of the monitor for about one second. We recorded evoked spiking activity in individual neurons by inserting a multichannel linear probe in V1 at the beginning of a behavioral session in trained mice. Although the probe spanned almost all layers of V1, the majority of units recorded during the task were found in the deeper layers of V1. After the behavioral session ended, we presented circular patches of gratings rotated at various angles. We found that units in the upper layers of V1 preferred orientations close to zero degrees. This preference, together with their low spontaneous firing rate, explained why units in the upper layers of V1 were not very responsive during the task. These results represent a first step in dissecting the role of visual cortical circuits in perceptual decisions.

Disclosures: **A. Resulaj:** None. **S.R. Olsen:** None. **M. Scanziani:** None.

Poster

434. Striate Cortex Plasticity I

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Support: Wellcome Trust 095668

Wellcome Trust 095669

Simons Foundation 325512

Title: Recordings from 10,000 neurons reveal high dimensionality in cortical activity

Authors: *M. PACHITARIU, C. STRINGER, S. SCHRODER, M. CARANDINI, K. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: Neural circuits impose constraints on the possible patterns a population of neurons can express. Understanding these constraints would provide insight into the underlying computations, but such understanding has been limited by the small size of recorded populations, which involve at most a few hundred neurons.
To overcome this limitation, we devised a calcium imaging protocol to record ~10,000 excitatory neurons simultaneously and we applied it to the visual cortex of awake head-fixed mice. The protocol involved imaging 10 planes (1 x 1 x 0.3 mm) at 3 Hz in mice expressing the calcium indicator GCaMP6s in selected neural populations, and identifiying cells with a novel cell detection algorithm (github.com/cortex-lab/Suite2P). Many of our findings would not have been possible by recording fewer (e.g. 300) neurons.
We found that spontaneous neuronal activity occupies a "spontaneous subspace" of at least a few hundred dimensions. The two leading singular vectors of this activity aligned with the average population rate and with fluctuations in arousal, respectively. Fluctuations in arousal, indicated by running or by pupil dilation, switched between two large populations of neurons that were spatially interspersed, while leaving the total firing rate mostly unchanged. The other dimensions reflected sparse sets of neurons and a variety of spatial and temporal timescales, from a few hundred microns to the entire 1 mm2 recorded surface, and from a few hundred milliseconds to tens of seconds.
To investigate the relationship of stimulus-driven responses to spontaneous activity, we presented sets of natural images while recording the same populations. Trial-averaged visual responses spanned a "stimulus subspace" approximately orthogonal to the spontaneous subspace. Trial-to-trial variability in population responses was dominated by fluctuations in the spontaneous subspace, but also contained substantial power in the stimulus subspace. Furthermore, the variability extended along dimensions of activity corresponding to all stimuli in our assay, not just those of the presented stimulus as would be expected from a multiplicative variability model.
We conclude that the large-scale structure of spontaneous activity is organized into a few hundred dimensions with varying spatial and temporal scales. Stimulus response patterns inhabit a different high-dimensional space with its own trial-to-trial variability. The large, internally-generated variability along this response subspace provides a possible substrate for the subjective, internally-biased interpretation of visual scenes demonstrated in human psychophysical experiments.

Disclosures: **M. Pachitariu:** None. **C. Stringer:** None. **S. Schroder:** None. **M. Carandini:** None. **K. Harris:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: NIH Grant EY021580

Saban Research Institute Intramural Predoctoral Award

Title: The recovery of ocular dominance and visual acuity in murine amblyopia are limited by NgR1 in distinct components of the visual circuitry

Authors: *C.-E. STEPHANY[1], H. M. DORTON[2], A. W. MCGEE[1];
[1]Children's Hosp. of Los Angeles, Los Angeles, CA; [2]USC, Los Angeles, CA

Abstract: The brain is profoundly impacted by life experience during development, especially during specified 'critical periods' early in life. In the visual system, abnormal vision during the critical period induces amblyopia, also known as "lazy eye". This common visual disorder is characterized by poor binocular vision and diminished acuity. The closure of this critical period limits recovery from amblyopia by inhibiting developmental plasticity and consolidating existing neural circuitry. In mice, the neuronal Nogo-66-receptor (NgR1) is required to close the critical period. NgR1 mutants display developmental visual plasticity as adults, and exhibit spontaneous recovery of ocular dominance as well as visual acuity in a mouse model of amblyopia, long-term monocular deprivation (LTMD). Yet, it is unclear if or how the recovery of these two forms of visual function is linked. Here, we demonstrate that the recovery of binocularity and visual acuity in murine amblyopia are distinct. Interestingly, deleting NgR1 only in thalamus and layer 4 of cortex yields a complete recovery of visual acuity, but does not rescue ocular dominance. In contrast, deleting NgR1 in all cortical excitatory neurons does not improve visual acuity following LTMD. Thus, NgR1 operates within the thalamocortical component of visual circuitry to regulate the capacity of experience to improve acuity.

Disclosures: C. Stephany: None. **H.M. Dorton:** None. **A.W. McGee:** None.

Poster

434. Striate Cortex Plasticity I

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DARPA W91NF-14-1-0269

SIMPLEX N66001-15-C-4032

MURI W911NF-12-1-0594

Title: Reprograming of neuronal ensembles in primary visual cortex with two-photon optogenetics *In vivo*

Authors: *L. CARRILLO-REID[1], W. YANG[2], D. PETERKA[2], R. YUSTE[2];
[1]Biol. Sci., [2]Columbia Univ., New York, NY

Abstract: Cortical ensembles in primary visual cortex are groups of neurons with coordinated activity that represent specific features of visual stimuli. The functional connectivity of these ensembles gives rise to activity patterns that generate an internal representation of the surrounding world. In this way neuronal ensembles recalled by sensory stimulation make use of imprinted patterns of activity stored in the cortical circuitry. However, whether it is possible to imprint and recall artificially created neuronal ensembles has been difficult to investigate. We used simultaneous two-photon optogenetics and imaging of neuronal populations in vivo to activate specific groups of neurons termed photoensembles whose members can be identified and manipulated with single cell resolution. Recurrent activation of the same photoensembles imprinted them in cortical microcircuits. Once established, single neuron photostimulation is able to recall a complete ensemble. Artificially imprinted photoensembles alternate their activity with visually evoked ensembles and can be used to alter mice behavioral tasks. Our findings demonstrate the possibility to reprogram ad libitum neuronal ensembles and observe the behavioral correlate of changes in the functional connectivity of cortical microcircuits.

Disclosures: **L. Carrillo-Reid:** None. **W. Yang:** None. **D. Peterka:** None. **R. Yuste:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: R01 EY022122

K01MH101639

R01NS065856

Charles Hood Foundation

Title: Rem2 is required for normal critical period ocular dominance plasticity of the visual cortex.

Authors: *S. E. RICHARDS, A. R. MOORE, K. KENNY, S. PARADIS, S. D. VAN HOOSER;
Neurosci., Brandeis Univ., Waltham, MA

Abstract: The fact that sensory experience contributes to the development of cortical circuits is well-established in the visual system. However, the identity of molecules responsible for transducing visual experience into proper development, maintenance, and plasticity of synaptic connections remains unclear. Recent work has suggested that the small GTPase Rem2 is a candidate molecule in this process. Rem2 is a known positive regulator of spine and synapse formation. Additionally, Rem2 function is regulated by CaMKII phosphorylation, placing Rem2 downstream of this critical molecule for long term potentiation (LTP). Previous work has also placed Rem2 in a signaling cascade upstream of CaMKIV, a proposed regulator of homeostatic plasticity. Furthermore, Rem2 expression is upregulated following brief exposure to light in the *Xenopus Laevis* optic tectum and mouse primary visual cortex, indicating that Rem2 expression can be altered *in vivo* by visual experience. Taken together, these results suggest that Rem2 has the potential to play a role in both Hebbian and homeostatic mechanisms that govern proper development and plasticity of visual circuits.
Here, we tested the hypothesis that Rem2 is necessary for activity-dependent changes in functional circuit responses to sensory stimulation in mouse visual cortex. We generated transgenic mice harboring a Rem2 null allele ($Rem2^{-/-}$) or a Rem2 conditional knockout allele ($Rem2^{flx/flx}$) and investigated critical period ocular dominance plasticity (ODP) induced by monocular lid suture, an extensively-studied cortical plasticity paradigm. ODP is characterized by a decrease in visual cortical responsiveness to stimulation of a deprived eye followed by an increase in responsiveness to input from the spared eye. We find that $Rem2^{-/-}$ mice show only a

partial ocular dominance shift, resulting primarily from a failure to reduce responsiveness to deprived eye input. This suggests a deficiency in the initial, Hebbian plasticity that results in decreased responsiveness to the obscured eye. Furthermore, by crossing cell type-specific Cre driver lines to our $Rem2^{flx/flx}$ line, we find that Rem2 is specifically required in excitatory pyramidal cells of the cortex, but not Parvalbumin-positive interneurons, for normal ODP to occur. Ongoing experiments aim to investigate the involvement of Rem2 in both Hebbian and homeostatic plasticity mechanisms required for ODP.

Disclosures: **S.E. Richards:** None. **A.R. Moore:** None. **K. Kenny:** None. **S. Paradis:** None. **S.D. Van Hooser:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: NIH R01 EY018441

Title: A surprising transient period of synaptic imbalance exists in the rat visual cortex during postnatal development

Authors: H. ZHANG[1], *M. T. WONG-RILEY[2];
[1]Cell Biology, Neurobio. and Anat., [2]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The mammalian visual cortex is not mature at birth, but undergoes a period of structural and functional adjustments. As abnormal development can lead to a host of visual impairment, it is important to take an in-depth look at the normal process of synaptic development. The goal of the present study was to record spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs) in the visual cortex of normal Sprague-Dawley rat pups *daily*, from the time of eye opening (P14) to P36, when neurons have presumably reached their maturity. We found, to our surprise, that the development of excitatory and inhibitory synapses did not follow straight, smooth paths. Instead, they exhibited three periods of development during this time: (1) From P14 to P27, the amplitudes of both sEPSCs and sIPSCs assumed a plateau, whereas the frequencies of both exhibited a gradual rise; (2) from P28 to P33, both the amplitudes and frequencies of sEPSCs fell precipitously and significantly below those of the previous period, whereas the amplitudes and frequencies of sIPSCs rose suddenly and significantly above those of the earlier period; and (3) from P34 to P36, both the amplitudes and frequencies of both sEPSCs and sIPSCs resumed the same, respective levels as those at P27.

These unexpected, striking results indicate that the visual cortex undergoes a drastic synaptic readjustment during the fifth postnatal week in the rat. This period falls within the critical period of the rat's visual system development.

Disclosures: H. Zhang: None. **M.T. Wong-Riley:** None.

Poster

434. Striate Cortex Plasticity I

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Topic: D.06. Vision

Support: Natural Science Foundation of China grant 31470975 (JYZ).

Natural Science Foundation of China grant 31230030 (CY)

Title: Dichoptic perceptual training in juvenile amblyopes with or without patching history

Authors: *J.-Y. ZHANG[1], X.-Y. LIU[2], C. YU[1];
[1]Peking Univ., Beijing, China; [2]Tengzhou Central People's Hosp., Tengzhou, China

Abstract: Dichoptic training is becoming a popular tool in amblyopia treatment. Here we investigated the effects of dichoptic training on juvenile amblyopia no longer responsive to patching treatment (PT group) or never patch treated (NPT group).
Training consisted of three stages. (1) 10 PT and 10 NPT amblyopes (8-17 years, 15-anisometropic, 3-ametropic, 1-strabismus, 1-mixed) received dichoptic de-masking training for 40 hours. They used AEs to practice contrast discrimination of Gabors that were dichoptically masked by a band-filtered noise pattern simultaneously presented in NAEs. Dichoptic learning is indexed by the increase of maximal tolerable noise contrast (TNC) for AE contrast discrimination. Training improved maximal TNC by 350% in PT and 480% in NPT, which translated to stereoacuity improvements by 4.6-lines in PT and 3.0-lines in NPT, and AE visual acuity improvements by 1.3-lines in PT and 2.1-lines in NPT. (2) The amblyopes further received stereopsis training for another 40 hours. Training improved stereoacuity by 2.4-lines in PT and 0.5-lines in NPT, and AE acuity by 0 line in PT and 0.5 lines in NPT. Seven PT amblyopes regained normal stereoacuity (20~50 arcsec) after two stages of training. (3) Extra monocular AE grating acuity training (30 hours) failed to improve grating acuity in both groups. Neither did it produce further AE acuity and stereoacuity gains. After training the visual acuity and stereoacuity gains persisted for at least one year.
Dichoptic training improved vision in both PT and NPT juvenile amblyopes. The PT group with

milder amblyopia benefited substantially more in stereoacuity (7.0-lines), probably because improved AE acuity (1.3-lines) could translate to greater balance of binocular vision and thus better stereoacuity. The NPT group benefited more in visual acuity (2.6-lines), consistent with our previous report (Liu et al., 2011). Our study confirmed the effectiveness of dichoptic training approaches in the treatment of juvenile amblyopia.

Disclosures: J. Zhang: None. **X. Liu:** None. **C. Yu:** None.

Poster

435. Cortical Planning and Execution: EEG

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: This research was supported by the intramural research program of the NIH Clinical Center (protocol #13-CC-0110).

Title: EEG/fNIRS as biomarkers for neural activation in humans during motor tasks

Authors: *T. H. CRUZ[1], T. BULEA[2], T. S. MOULTON[3], A. C. DE CAMPOS[4], A. SHARMA[2], T. HUPPERT[5], D. DAMIANO[2];
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Abstract: The ability to combine mobile neuroimaging techniques, e.g. electroencephalogram (EEG) and functional near-infrared spectroscopy (fNIRS), during a movement task could improve mapping of brain activation underlying motor activity. These techniques have complementary strengths and weaknesses: EEG has high temporal resolution but low spatial resolution due to volume conduction; whereas fNIRS has low temporal resolution due to the hemodynamic response, but higher spatial resolution. The challenges in combining these methods are both physical and computational. To this end, we compared brain activation using EEG and fNIRS during movement by 9 healthy volunteers (4 M; 26 ± 7 yrs, 8 right-handed) under an IRB-approved protocol. Each performed unilateral lower limb tasks with the dominant leg (ankle dorsiflexion and cycling) on two separate days, with the order of EEG and fNIRS randomized. 64-channel EEG was collected using a wireless active-electrode system (Brain Products, Morrisville, NC) with electrodes placed according to the 10-20 system. fNIRS (TechEn, Inc., Milford, MA) was recorded from 44 channels (8 sources & 16 detectors) placed in

a custom probe (Moulton et. al, 2014) optimized to detect signals from primary motor and dorsal premotor cortices. Motion capture was used to measure movement and to co-register locations of the EEG and fNIRS probes. EEG and fNIRS data were processed using EEGLAB (Delorme and Makeig, 2004) and the +NIRS Toolbox (Huppert, 2016), respectively. We examined traditional measures of cortical activation from each modality, i.e. event-related potentials and spectral perturbations from EEG and changes in oxygenated (HbO) and de-oxygenated (HbR) hemoglobin from fNIRS. The fNIRS data, analyzed in block averages, showed significant increases in HbO and decreases in HbR in the contralateral medial motor cortex, as expected for a unilateral lower limb task. Changes in HbO and HbR scaled with the difficulty of the task. In the EEG, movement-related changes were most prominent along midline motor areas, with a more diffuse presentation in the multi-joint cycling task. Interestingly, cortical areas displaying neurovascular coupling during blocks of fNIRS were correlated with EEG channels showing activation on a much faster timescale. Our results demonstrate the potential of multi-modal, portable imaging as a biomarker for localizing neural activity during movement. Such biomarkers are promising for identification of cortical regions underpinning movement in individuals with brain injury, many of whom are unable to remain still for fMRI, because these could provide well-defined, individualized targets for neurorehabilitation.

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Poster

435. Cortical Planning and Execution: EEG

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 435.02/TT3

Topic: E.04. Voluntary Movements

Support: Mazda Foundation

Title: EEG signals related to movement-related cortical potential by direction-cue and go-cue

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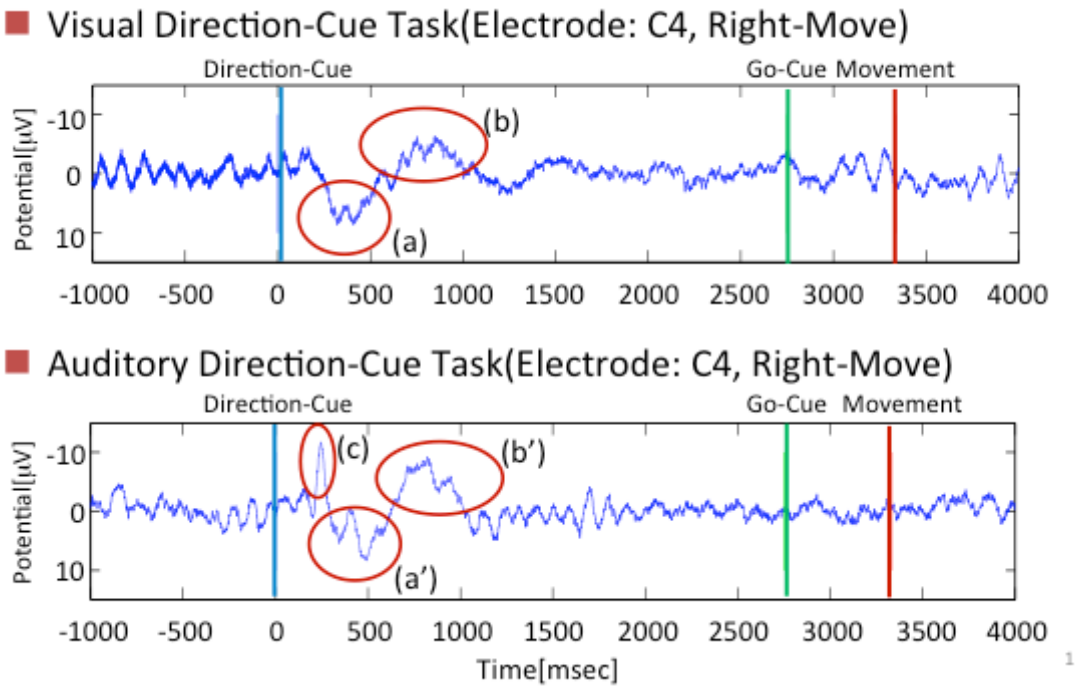
Abstract: [Purpose] We have been studying the movement-related cortical potential on voluntary movements in the point of temporal patterns of brain activity. In this research, we perform the experiments using by the Direction-Cue and the Go-Cue (this experiments are called “Direction-Cue task”). We can distinguish between the brain activity of decision-making related to direction of movements and the brain activity of connecting between the direction-cue and

movements and the brain activity of preparation on movements. [Experiment] We perform the auditory direction-cue task and the visual direction-cue task. The direction-cue show the direction of movements and the go-cue show the start timing of movements by a beep sound. Experimental order in the visual direction-cue task is as following. 1) A subject watches a fixation point on a computer display. 2) After 4.5-6.0 [sec], a direction-cue is presented to a subject. 3) After 2.5-3.0 [sec], a go-cue is presented to a subject. 4) A subject pushes a bottom. When the direction-cue is shown as the right-arrow on a computer display, a subject pushes a right-side bottom. When the direction-cue is shown as the left-arrow, a subject pushes a left-side bottom. In the auditory direction-cue task, direction-cues are shown as a beep sound. When the beep sound is shown from a right hand side speaker, a subject pushes a right-side bottom. When the beep sound is shown from a left hand side speaker, a subject pushes a left-side bottom. This order is one trial flow. We perform 50 right trials and 50 left trials. We record the EEG signals during these tasks. We focus on the C3, C4, Cz electrode. EEG signals are processed by the ensemble averaging. On-set of the ensemble averaging is at starting time of the direction-cue. [Results and Discussion] From a figure, we obtain two features (a), (b) in the visual direction-cue task and three features (c), (a'), (b') in the auditory direction-cue task. The (c) is a feature related to understanding auditory stimuli. The (a) and (a') are related to decision-making of movement-direction. The (b) and (b') are related to preparation of movements.



Disclosures: **A. Funase:** None. **S. Takagi:** None. **I. Takumi:** None.

Poster

435. Cortical Planning and Execution: EEG

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Topic: E.04. Voluntary Movements

Support: NIH Grant 1R01HD086245

NSF Grant 1539067

Title: Children with cerebral palsy have uncharacteristic beta cortical oscillations during a visuomotor target matching task

Authors: *M. J. KURZ[1,2], A. L. PROSKOVEC[2], J. E. GEHRINGER[1,2], E. HEINRICHS-GRAHAM[2], T. W. WILSON[2];
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Abstract: The literature on cerebral palsy (CP) has predominantly focused on identifying the potential faults that exist within the musculoskeletal system, with little regard for aberrations that may exist within the key cortical networks that serve the uncharacteristic motor actions seen in these children. In this study, we interrogated the beta (15-30 Hz) cortical oscillations as a cohort of children with CP (N=13; Age = 15 $\pm$ 3 yrs; GMFCS levels II-III) and typically developing children (N=15; Age = 14 $\pm$ 3 yrs.) performed an isometric knee extension target matching task. The isometric forces generated by the child elevated an animated box vertically in order to match visual targets that were between 5-30% of the child's maximum force. The epochs of each trial were 10 seconds in duration (-5.0 s to +5.0 s), with the onset of the isometric force defined as 0.0 s. The reaction time, amount of target overshoot, and time to match the target were used to assess the child's motor performance. Magnetoencephalography (MEG) was concurrently collected to assess cortical oscillatory activity during the 120 target matching trials. The resulting MEG data was noise corrected, coregistered to the child's MRI, filtered and subjected to standard artifact rejection methods. A beamformer analysis was performed to image the beta oscillatory activity that was present during the motor planning (-500 to 0 ms) and motor action stages (0 to 500 ms). Our results showed that the children with CP had slower reaction times, a greater amount of overshoot, and took longer to match the targets (*Ps* <0.03). The children with CP also had a stronger beta event related desynchronization (ERD) during the motor planning stage in the SMA, left premotor cortices and primary motor cortices ($P < 0.01$, cluster corrected). During the motor action, the children with CP continued to have a stronger beta ERD within the SMA and left premotor cortices ($P <0.01$, cluster corrected). In addition, the children with CP had a weaker beta event related synchronization (ERS) in the left prefrontal, MT/V5 visual area, and bilaterally in the occipital cortices during the motor action ($P<0.01$; cluster corrected). These results indicate that the uncharacteristic motor actions seen in children with CP may partially be

related to abnormalities in neural computations that are involved in planning a goal directed motor action. Furthermore, movement in children with CP may be further compromised by deficient or inaccurate visuomotor transformations, which are necessary for matching a desired motor goal.

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Poster

435. Cortical Planning and Execution: EEG

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Program#/Poster#: 435.04/TT5

Topic: E.04. Voluntary Movements

Support: Wellcome Trust studentship

Title: Orthogonalising parameters of predictive coding within a visuomotor adaptation task

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Abstract: Perturbations introduced in visuomotor adaptation tasks produce prediction errors, which are used to update forward models to generate more accurate sensorimotor predictions. Prediction errors are precision-weighted meaning an estimate of the inverse variance of the afferent signal (sensory precision) and the motor prediction (prior precision) is used to determine how readily prediction errors update forward models. These parameters are modulated during adaptation however their neurophysiological correlates are not fully understood. It has been suggested that beta oscillatory activity over sensorimotor cortex encodes this Bayesian updating process (Tan et al, 2014, 2016; Torrecillos et al, 2015). However, within the paradigms studied it was difficult to dissociate prediction errors from their precision weighting. The current study uses a paradigm designed to orthogonalise prediction error, prior precision and sensory precision in order to identify whether beta oscillations are correlated with any of these parameters. In this study, EEG was recorded whilst 18 subjects completed a visuomotor adaptation task. Subjects were instructed to move a cursor from a start position into a target. Perturbations in the visual feedback of the cursor forced subjects to generate online corrective movements to reach the target. Each perturbation remained the same for 10-15 trials to allow for adaptation. Certainty in motor predictions (prior precision) was modulated by altering the probability with which the next trial would require the same movement; this pattern was dissociable from that of prediction error

modulation. Sensory precision was also orthogonally modulated by introducing random error into the position of the cursor on the screen in a block design; adding noise into the sensory input made the signal more uncertain and decreased sensory precision in noisy vs non-noisy blocks. EEG data in the time period before trial onset was analysed to determine the state of the system prior to moving and after each trial to examine the role of the PMBS in adaptation. Each parameter was modelled using the behavioural data to determine what activity within the time-frequency domain overlying the sensorimotor cortex correlated with each of the different parameters. Differences in alpha (8-12Hz), low beta (13-18Hz) and high beta (19-30Hz) were found for the parameters of prior precision, sensory precision and prediction error. This study has implications for understanding how predictive coding is encoded in the brain and can help reframe disorders in which these correlates are dysfunctional to provide a new insight for the development of novel therapies.

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Poster

435. Cortical Planning and Execution: EEG

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Topic: E.04. Voluntary Movements

Support: JSPS Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers

MEXT/AMED SRPBS (BMI)

MEXT KAKENHI 26112004

Title: Directionally tuned signals in human EEG during step-tracking wrist movement

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Abstract: Many electrophysiology experiments in monkeys have long contributed to identifying neural activities that covary with parameters during directional arm and wrist movements. On the other hand, it remains largely unexplored the neural mechanisms of directional movements in humans, and the newly developing area of Mobile Brain/Body Imaging (MoBI) has a promise of

noninvasive recording of humans during unconstrained body movements. In this study, we report that directionally tuned neural activities during step-tracking wrist movement can be captured by high-density EEG signals during arm reaching movements
Subjects were seated on a chair with their forearm held in one of three postures (full-pronation, full-supination, or midway between the two positions) and was instructed to make step-tracking wrist movements from the neutral posture to eight different targets that required varying amounts of flexion-extension and radial-ulnar deviation. Neural activities and wrist joint kinematics were monitored in synchrony with 128 channel EEG and a motion capture.
Independent component analysis and equivalent dipole fitting of resulting brain-based independent component (IC) scalp maps were applied to the EEG data and revealed cortical current sources in visual, somatosensory, and motor cortical areas. We computed temporal correlations between trial-by-trial movement-locked event-related potentials (ERPs) of each IC. For the ERPs within same postures, all of the brain-based ICs exhibited maximal correlation for pairs of trials with same movement direction. The correlation coefficient smoothly decreased with the increasing difference of movement directions. This correlation pattern was well fitted by a Gaussian function against difference in movement directions. This finding of structured correlations indicated that the cortical ICs were tuned to the wrist movement directions. Next, we computed temporal correlations of ERPs across different forearm postures. Whereas most ICs showed no shifts with respect to the forearm postures, some ICs exhibited the shift of correlation curves, i.e., ERPs of one direction in one posture correlated maximally with those of slightly shifted direction in another posture. We found ICs exhibiting the shift in maximal correlation consistent with the shift in preferred direction of neurons representing muscle activities found in Kakei et al. (1999).
The results of this study show that the neural activities related to movement parameters, such as movement direction and muscle activities, can be captured from high density EEG recorded during unconstrained body motion.

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Poster

435. Cortical Planning and Execution: EEG

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JSPS Grant-in-Aid 26120005

Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers

Title: Dynamics of directional tuning and reference frames in humans: A high-density EEG study

Authors: *H. TANAKA[1], M. MIYAKOSHI[2], S. MAKEIG[2];
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Abstract: Neural mechanisms controlling upper-limb reaching movements have been studied traditionally at the single-neuron level in monkey electrophysiological studies and, more recently, at the voxel level in human fMRI studies. However, the single-unit recording approach lacks a whole-brain perspective, and the fMRI approach misses sub-second neural dynamics and does not allow natural, unconstrained movements. This study exploits recent developments in EEG recording and signal processing to investigate neural computations underlying ballistic pointing movements. High-density EEG with more than 200 electrodes covering the entire scalp has near-msec temporal resolution and can be used to measure neural activities under unconstrained physical conditions, thereby resolving limitations of previous studies. We specifically asked how EEG source dynamics indexed finger movement directions (directional tuning) and posture dependence (movement reference frames) by applying representational similarity analysis. A majority of the EEG source processes studied exhibited statistically significant directional tuning in peri-movement periods, appearing before movement onset and disappearing after the movement termination. In addition, directional tuning curves shifted when the shoulder angle was rotated to perform the task within a more laterally positioned workspace, the degree of tuning curve rotation falling between that predicted by models assuming extrinsic and shoulder-based reference frames. We conclude that cortical dynamics engaged during motor control can be studied noninvasively in humans using high-density EEG and advanced signal processing, at a level matching results reported in single-cell electrophysiological studies using monkeys.

Disclosures: H. Tanaka: None. **M. Miyakoshi:** None. **S. Makeig:** None.

Poster

435. Cortical Planning and Execution: EEG

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Topic: E.04. Voluntary Movements

Support: NSF Grant 1539067

Title: Altered sensorimotor cortical oscillations in individuals with multiple sclerosis suggests a faulty internal model

Authors: *D. J. ARPIN[1,2], E. HEINRICHS-GRAHAM[2], J. E. GEHRINGER[1,2], T. W. WILSON[2], M. J. KURZ[1,2];
[1]Physical Therapy, [2]Ctr. for Magnetoencephalography, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Multiple sclerosis (MS) is a demyelinating disease that results in impaired motor performance. However, the neurophysiological changes responsible for these motor control problems remain unknown. Potentially, these movement errors may be due to imperfections in the internal model used to make predictions of the motor output based on the task demands. Prior magnetoencephalographic (MEG) and electroencephalographic (EEG) brain imaging experiments have established that beta (15-30 Hz) oscillatory activity in the sensorimotor cortices is related to the control of movement. Recent experimental work has also shown that changes in the beta-frequency activity at movement termination, termed post-movement beta rebound (PMBR), reflects the certainty of the feedforward motor actions that were executed based on an internal model. In this study, we used MEG to evaluate the neural oscillatory activity in the sensorimotor cortices of individuals with MS (N=13; Age = 57 $\pm$ 6.5 yrs; EDSS = 5.5 $\pm$ 0.7) and healthy age-matched controls (N=13; Age = 55 $\pm$ 7.3 yrs.) during a goal-directed knee extension force task. A custom built force transducer was attached to the subject's lower leg, providing a measure of isometric knee extension force. The forces generated elevated an animated box toward the target, and the participants matched targets between 5 and 30% of their maximum force. The epochs of each trial were 10 seconds in duration (-5.0 s to +5.0 s), with the onset of the isometric force defined as 0.0 s. Each participant performed 120 trials of the target matching task while reaction time, amount of overshoot, average velocity to the target, and time to match the target were recorded. MEG was used to record the oscillatory activity of the sensorimotor cortices as the subjects performed the target matching task. The MEG data were noise corrected, coregistered to MRI, filtered, and subjected to standard artifact rejection methods. A beamformer analysis was performed for the PMBR (16-26 Hz, 4.0 to 4.8 s) following movement termination. The grand average of the beamformer images was used to find the peak voxel, and the time series of this voxel was extracted in each participant and subjected to group-level statistical analyses. Our results showed that individuals with MS had slower reaction times ($p<0.01$), a greater amount of overshoot ($p<0.01$), slower average velocity to the target ($p<0.01$), and took longer to match the target ($p<0.01$). Individuals with MS also had a weaker PMBR in the pre/postcentral gyri relative to healthy controls. The aberrant PMBR suggests that the internal model is faulty in individuals with MS and potentially responsible for their uncharacteristic motor performance.

Disclosures: D.J. Arpin: None. **E. Heinrichs-Graham:** None. **J.E. Gehringer:** None. **T.W. Wilson:** None. **M.J. Kurz:** None.

Poster

435. Cortical Planning and Execution: EEG

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Program#/Poster#: 435.08/TT9

Topic: E.04. Voluntary Movements

Support: NIH NICHD 1R01HD086245

NSF 1539067

Title: Neuromechanical changes associated with learning an isometric ankle plantarflexion target matching task

Authors: *J. GEHRINGER[1], D. J. ARPIN[1], E. HEINRICHS-GRAHAM[2], T. W. WILSON[2], M. KURZ[1];
[1]Physical Threapy, [2]Ctr. for Magnetoencephalography, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: It is well accepted that the cortical oscillatory changes that occur prior to movement onset at the beta frequency (15-30 Hz) reflect the neural computations that are involved in planning motor actions. Moreover, a stronger beta event-related desynchronization (ERD) during the motor planning stage is associated with greater certainty that the selected motor action will meet the task demands. Still, we do not fully understand how these oscillations change as individuals learn a motor task. Nine healthy adults (Age = 23 ± 4 yrs.) practiced a discrete isometric ankle plantarflexion target matching task. The force production of the left ankle was collected with a custom-built force transducer. The generated force caused an animated a box to ascend, and the participants matched targets between 20 and 35% of their maximum force, which disappeared upon when the target was reached. The epochs of each trial were 10 seconds in duration (-5.0 s to +5.0 s), with the onset of the isometric force defined as 0.0 s. Each participant performed three 100-trial practice blocks. The cortical activity was recorded using magnetoencephalography (MEG) during the first and third blocks. Learning induced behavioral improvements were evaluated by comparing the reaction time, initial force production velocity, amount of overshoot, and amount of variability while trying to match the target between the first and third blocks. The MEG data were noise corrected, coregistered to MRI, filtered, and subjected to standard artifact rejection methods. The neural regions generating the beta ERD and time windows of interest were isolated and the data were subjected to beamformer analysis. Our results showed that the participants had behavioral improvements after practice. The reaction times were faster (P=0.017), initial force production velocity was faster (P=0.004), they had less overshoot (P=0.040), and less variability when trying to match the targets (P=0.015). These behavioral improvements paralleled the changes seen in the cortical oscillatory activity. The beta ERD ($P < 0.05$, cluster corrected) during the planning stage was stronger in the motor cortex, supplementary motor area, and frontal cortex after practice. The stronger beta ERD within these

respective areas suggest that the participants had greater certainty in selecting an isometric force that would match the prescribed targets. Altogether these results imply that changes in the strength of the beta ERD reflect the cortical changes that are associated with learning an ankle plantarflexion motor task.

Disclosures: J. Gehringer: None. **D.J. Arpin:** None. **E. Heinrichs-Graham:** None. **T.W. Wilson:** None. **M. Kurz:** None.

Poster

435. Cortical Planning and Execution: EEG

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 435.09/TT10

Topic: E.04. Voluntary Movements

Title: Improving source localization of movement-related potentials with tri-polar electroencephalography

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Abstract: Knowing where sources of activity are located in the brain can help with diagnosis. Locating the sources of electroencephalography (EEG) signals acquired on the scalp, the inverse problem, is an ill-posed problem since there are an infinite number of source configurations that can result in a particular potential map on the head surface. Therefore, additional constraints to the source space must be used to find a unique solution. Equivalent current dipole methods utilize a discrete source space in which a small number of dipoles are assumed to generate the given surface potential. Distributed source methods constrain the source space to a larger number of dipoles distributed either on the cortical surface or within the brain. Both methods have their advantages, but discrete source spaces yield an overdetermined solution while the solutions of distributed source methods are underdetermined.
In the present study, the increased spatial resolution of tri-polar EEG (tEEG) minimized the underdetermined nature of distributed source localization methods with respect to movement related potentials (MRPs). Subjects (n=5) were concurrently recorded with both conventional EEG and tri-polar concentric ring electrodes (TCREs) during periods of a visually cued, right index finger flexion task, a mouse click. Electrodes were placed on 16 of the standard 10-20 locations, including FP1, Fp2, F7, F3, Fz, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P8, and O1. Surface potentials related to index finger flexion were averaged per subject and subsequently localized using distributed source methods on the ICBM152 head model derived from a non-

linear average of MRI scans of the 152 subjects in the MNI152 database. Using the open-source data analysis application Brainstorm, a linear L2-minimum norm estimates algorithm was used to localize sources to a source space of dipoles constrained normal to the cortical surface, and subject results were normalized to Z-scores before group analysis. The locality of activated brain regions was then compared between conventional EEG and tEEG results.
Localization results obtained from tEEG appeared to be much more focal when compared to those of conventional EEG. Thus, the underdetermined nature of distributed source localization methods is decreased when used with tEEG, alleviating this drawback and producing a more accurate representation of the MRP source signal.

Disclosures: C. Toole: None. **P. Steele:** A. Employment/Salary (full or part-time): CREmedical. **J. DiCecco:** None. **W. Besio:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CREmedical.

Poster

435. Cortical Planning and Execution: EEG

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Title: Dynamic phase-amplitude coupling in the EEG during gait adaptation

Authors: *J. WAGNER[1,2], R. MARTINEZ CANCINO[2], C. NEUPER[3], G. MUELLER-PUTZ[1], S. MAKEIG[2];
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Abstract: Successfully performing rhythmic movements including walking synchronized to a rhythmic cue sequence, exact temporal prediction and motor execution are essential. It has been proposed that synchronous entrainment of brain activity to external pacing events may play a role in supporting temporal motor regularity. This entrainment has been suggested to optimize timing of action execution by controlling excitability in sensory cortices. In previous work (Wagner et al., 2012, 2014) we have shown that low gamma band activity in the Supplementary Motor Area (SMA, a critical motor planning node) entrains to the gait cycle during steady-state treadmill walking, suggesting a role of these oscillations in the planning of rhythmic movements. To investigate the role of cortical field oscillations in the temporal planning of gait, we performed a study in which we examined the high-density EEG dynamics of participants attempting to step in time to an auditory pacing tone sequence. After periods of steady-state walking, participants had to adapt their step length and rate to shifts in tempo of the pacing stimulus (e.g., following unforeseen shifts to a faster or slower pacing tempo) (Wagner et al., 2016). Analysis revealed that during steady-state walking, cortical field activity projecting to the scalp from one or more sources isolated in or near the SMA entrain to the rhythm of walking in both the (25-40 Hz) low gamma and (4-8 Hz) theta frequency bands, suggesting cross-frequency coupling between these rhythms. To assess this, we tested for coupling between low gamma and theta band activities by testing for coupling between all combinations of phase and amplitude in the two frequency bands. We found that during steady-state walking, ~35-Hz low-gamma band power amplitude is coupled to ~4-Hz theta phase. This coupling is maximal during stance and swing gait cycle phases, but is absent during transitions between these movement phases. We propose that this entrainment and coupling of theta and low gamma oscillations may help generate temporal predictions required for maintaining the temporally precise synchronization of gait to external pacing events.

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Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI Grant 23650335

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Title: Changes associated with motor learning of TMS-evoked EEG responses

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Abstract: Humans can perform accurately and quickly the intended movement with repeated training in daily living. The purpose of this study was to reveal the changes of neural network in the brain associated with motor learning by combining the electroencephalographic (EEG) recordings of the brain activity with transcranial magnetic stimulation (TMS). Eight healthy subjects (mean±S.D., 22.6±3.8 years, male n=6, female n=2) with no history of neurological disorders participated in the present study. All subjects were right-handed. Subjects were instructed to press four numeric keys on a standard computer keyboard with the fingers of their left hand (non-dominant), repeating the five-element sequence, 1-2-4-3-1, as accurately and as quickly as possible, for a period of 30 sec. One single training session consisted of 12 sets, 30-sec trials with 30-sec rest periods between trials. EEG signals were recorded using a flexible electrode cap with 16 electrodes (AFZ, F3, FZ, F4, FC1, FC2, T7, C3, CZ, C4, T8, CPZ, P3, PZ, P4, and OZ). During each rest period, TMS was applied to the scalp 6 times in inter-stimulus interval at 5 sec (72 stimulus in one session). TMS application carried out 3 times, at first session, 1 h after the first session (second session), and 1 week later (third session). Subjects trained three times per week, with each training session being comprised of 12 sets. A figure-of-eight coil was held over the right motor cortex (at the optimum scalp position to elicit motor responses in the contralateral FDI) with the induced current flowing in a postero-anterior direction, and was rotated away from the mid-line by up to 45º. The focal point was defined as the lowest threshold site giving a response specifically in the FDI muscle at rest. Stimulus intensity was at 60 % of the resting motor threshold. Performance was evaluated as the skill index (= % correct sequences / mean correct sequence time per each 30 s trial). The skill index was significantly increased with training ($P < 0.05$). We analyzed an amplitude of evoked potentials (N100 and P200), an event-related power (ERPow: the amount of change for mean power), and Phase-locking index (PLI: the extent of phase synchronization between trials). The latter two indexes are derived from a frequency analysis. There was no difference for amplitude of the evoked potentials (N100 and P200) among 3 sessions. However, the number of channels in ERPow and PLI associated with performance were increased in the frontal area of the brain in the third session. These results suggested that neural activity will be efficient in the frontal cortex with motor training.

Disclosures: **H. Sekiguchi:** None. **S. Takeuchi:** None. **M. Miyazaki:** None. **K. Yamanaka:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 436.02/TT13

Topic: E.04. Voluntary Movements

Support: Kakenhi (25119001)

Title: Independent preparation of “what” and “when”in the cortico-spinal pathway.

Authors: *N. HAGURA[1], Y. GOTO[2], M. MATSUMURA[2];
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Abstract: Preparation of action requires both spatial (i.e. which action) and temporal (i.e. when to move) aspects. Here, we show that these two information are concurrently processed during the preparation period, each independently modulating the motor system. 14 subjects participated in the study. In a trial, an arrow indicating either left of right was presented on a monitor (GO signal). Subjects flexed their right wrist for the left arrow and extended for the right in a ballistic manner. In each block, the presentation ratio of left and right arrows varied (7:1, 3:1, 1:1, 1:3, 1:7), enabling the subjects to estimate which movement (flexion or extension) is more likely to be performed during that block. In each block, for half of the trials, a warning signal was presented 500ms before the GO signal. This allowed the subjects to predict the initiation timing of the movement. To evaluate the cortico-spinal excitability during motor preparation, single pulse transcranial magnetic stimulation (TMS) was applied to the left primary motor cortex 50~500ms before the GO signal, and motor evoked potentials (MEP) were measured from *flexor carpi radialis* (FCR) and *extensor carpi radialis* (ECR). Reaction time (RT) was reduced both by the information of the movement type and the movement timing, without showing any effect of interaction. This indicates that two information independently act on the motor system for efficient motor preparation. This behavioural pattern of independent information processing was similarly reflected on the MEP. Overall amplitude increased depending on the probability of the movement type (effect of movement type), but on top of that, reduction of MEP towards the GO signal was observed when the warning signal was presented (effect of movement timing). We propose that the information about movement types and movement timings are concurrently processed, possibly in different brain regions, acting on the cortico-spinal pathway from the motor cortex at different stages.

Disclosures: **N. Hagura:** None. **Y. Goto:** None. **M. Matsumura:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: R01 NS074917

Title: The gradient level of inhibition in response preparation

Authors: *L. LABRUNA[1], C. TISCHLER[1], D. LEVITIN[2], M. J. DABIT[1], I. GREENHOUSE[1], F. LEBON[3], R. B. IVRY[1];
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Abstract: Cortical and subcortical inhibitory mechanisms help to sharpen the preparatory processes for a specific action, ensuring efficient performance. Relative to the selected effector, there is a gradient of inhibition such that some effectors show greater inhibition than others. This gradient could be anatomical, reflecting the distance between cortical representations of the body, functional, reflecting the history of interaction between different effectors, or a combination of factors. For example, compared to the foot, inhibition of the pinky could be greater when preparing to move the index finger either because the two fingers have neighboring cortical representations or because the two fingers are often involved in coordinated actions. In two experiments we used transcranial magnetic stimulation (TMS) to probe the state of corticospinal excitability to explore these hypotheses. In a first experiment, we compared the levels of inhibition during preparation of movements of effectors with cortical representations of variable distance relative to a probed muscle. In a second experiment, we tested professional drummers to see if they showed a different pattern of graded inhibition compared to control participants, given their history of extensive interactions between upper and lower limbs.
TMS was used to elicit motor-evoked potentials (MEPs) of the left index or left foot during a delayed response task. Each trial consisted of a fixation marker, a preparatory cue indicating the response for the forthcoming trial, and 900 ms later, an imperative signaling that the prepared response should be initiated. A single TMS pulse was applied over the right M1, either at the onset of the fixation (baseline) or before the imperative signal (delay). The results showed that MEPs were always reduced at the end of the delay period, relative to baseline, confirming the engagement of inhibitory mechanisms during motor preparation. Consistent with the anatomical hypothesis, the strength of inhibition was graded as a function of the proximity of the cortical representation of the body part involved in the prepared action to the probed muscle. The pattern of inhibition was similar in drummers and control participants, challenging the hypothesis that

the graded inhibition in different body parts is functionally related to the history of performing coordinate actions.

Disclosures: **L. Labruna:** None. **C. Tischler:** None. **D. Levitin:** None. **M.J. Dabit:** None. **I. Greenhouse:** None. **F. Lebon:** None. **R.B. Ivry:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

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Program#/Poster#: 436.04/UU1

Topic: E.04. Voluntary Movements

Support: CIHR

Title: Examining the influence of dorsolateral prefrontal cortex activity on ipsilateral primary motor cortex excitability with dual-site TMS

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Abstract: Objective: Frontal cortical areas interact for action planning and decision-making. The influence of the dorsolateral prefrontal cortex (DLPFC) activity on ipsilateral primary motor cortex (M1) excitability has not been systematically investigated.
Methods: Connectivity between DLPFC and M1 was examined using paired-pulse transcranial magnetic stimulation (TMS) using two coils in 12 right-handed participants (aged 21-44 years). Both right and left hemispheres were tested in separate sessions. Motor evoked potentials (MEPs) were recorded using surface electromyography from the first dorsal interosseous (FDI) and abductor pollicis brevis (ABP) muscles. The test stimulus (TS) was applied to M1 to induce MEP amplitude of ~1mV with both hands relaxed (Rest Condition) and while individuals held a 10% maximum voluntary contraction (Active condition). DLPFC was localized according to individual anatomical landmarks on MRIs along the middle frontal gyrus (BA 46). The conditioning stimulus (CS) was applied to DLPFC at stimulation intensities of 80% and 120% resting motor threshold (RMT). CS was applied before TS at inter-stimulus intervals (ISI) of 4-12, 15 and 20 ms.
Results: Despite individual variability, conditioning stimulation to the left DLPFC increased MEP amplitudes in ipsilateral M1 at two phases: an early peak between 5-8 ms and later peak at 9-11 ms. This facilitation was more prominent in the APB muscle compared to the FDI muscle. Rest condition with CS 80%RMT tended to produce greater facilitation compared to the other conditions. Interactions between right DLPFC and right M1 were more variable than the left

side.
Conclusion: These findings may provide effective parameters to evaluate DLPFC-M1 connectivity for sensorimotor actions in healthy, injured, and diseased brains.

Disclosures: M.J. Brown: None. **M. Vesia:** None. **C. Gunraj:** None. **R. Chen:** None.

Poster

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Program#/Poster#: 436.05/UU2

Topic: E.04. Voluntary Movements

Title: Cortical processes to predict timing of gait initiation through visual information

Authors: *K. TAKEDA[1], Y. NISHI[3], H. MANI[2], N. HASEGAWA[1], T. ISHIGAKI[3], Y. TAKAMURA[3], M. OSUMI[4], S. NOBUSAKO[4], H. MAEJIMA[2], S. MORIOKA[4], T. ASAKA[2]; [1]Grad. school of Hlth. Sci., Hokkaido Univ., Sapporo-Shi, Japan; [2]Dept. of Rehabil. Science, Fac. of Hlth. Sci., Hokkaido Univ., Sapporo-shi, Japan; [3]Dept. of Neurorehabilitation, Grad. Sch. of Hlth. Sci., [4]Neurorehabilitation Res. Ctr., Kio Univ., Kitakatsuragi-gun, Japan

Abstract: Introduction
We predict the clearance of other people spatiotemporally based on visual information to prevent collision in the crowd. As far as we know, no study has investigated the brain activities for timing control during gait initiation. The purpose of this study was to clarify the cortical processes related to timing control of gait initiation through visual information by electroencephalogram (EEG).
Methods
Thirteen healthy young adults participated in this study. Participants stood in front of a screen on which a horizontal moving target and a fixed goal were projected. They were instructed to synchronize the gait initiation when the target arrived at the goal on the screen. The participants were able to watch the target until it arrived at the goal under the control condition. On the other hand, the target vanished from 0.6 or 0.8 s before the target arrived at the goal under the vanishing condition, which was based on a previous study concerning motor preparation (Jacobs, 2008). The task consisted of 30 trials in each condition. Variable error (VE), which was the standard deviation of the temporal differences between the time when the target arrived at the goal and the initiation time of the heel-off, was calculated to assess the regularity of timing control. We measured brain activities by EEG and mainly focused on the power in the frontal region and the phase synchronization between the frontal and parietal regions in theta band (4-7Hz) during the target moving.

Results and Discussions

VE across the first 10 trials under the vanishing condition was significantly higher than that under the control condition. However, there were no significant differences between the conditions across 10 trials both of the middle and the last sessions of the 30 trials. It was suggested that the participants controlled the timing independently from the visual information in the middle and last sessions. Therefore, the first 10 trials were selected to analyze brain activities. At approximately 0.5 s prior to the arrival of the target at the goal, the power and the phase synchronization under the vanishing condition were significantly lower than those under the control condition. Furthermore, the synchronization showed significantly negative correlation with VE. We conclude that the connectivity between posterior parietal and frontal region at approximately 0.5 s before the predicted time of the motor execution is essential to control the timing of gait initiation based on visual information. Our results would be basic knowledge for prevention of collision and intervention for gait disorder, such as freezing, in the crowd.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH/ NICHD grant 1R01HD071978-01A1

Title: Gaze pattern differences inform hand posture to object shape during reach-to-grasp

Authors: *A. YOUSEFI[1], S. BILALOGLU[1], J. R. RIZZO[1], Y. LU[2], P. RAGHAVAN[1,2]; [1]Rehabil. Med., New York Univ. Sch. of Med., New York, NY; [2]Steinhardt Sch. of Culture, Education, and Human Develop., New York, NY

Abstract: Every day we grasp numerous objects of various shapes. Visual perception of object shape informs the planning of hand posture for an efficient grasp. A clear understanding of eye-hand coordination during adaptation of hand posture to object shape is necessary to develop appropriate treatment strategies for patients with hand function impairment. This study explores the relationship between gaze location and hand position during reaching to grasp objects of various shapes in healthy subjects. Eight neurologically intact, right-hand dominant subjects participated in the study; five were right eye-dominant and three were left eye-dominant. The

subjects sat at a table with the grasping hand palm-down in front of them, and then reached towards and grasped an object positioned at 75% of their arm length in front of them. The task was repeated seven times for nine different object shapes. The gaze vectors related to the two eyes were tracked using Eyelink2 eye tracker (SR Research Ltd, Ottawa, Ontario, Canada), and the location of the subject's head, hand and fingers were tracked by motion sensors attached to the limb segments and to the objects (6DOF Ascension sensors, Ascension Technology Corporation Shelburne, Vermont). The Cyberglove (Cyberglove system, San Jose, USA) was used to measure finger joint angles to compute hand posture. We examined the intersection of the gaze vector with the object for each eye during various phases of reach-to-grasp: upon eye opening (pre-reach), from reach onset to peak velocity (reach acceleration), from peak velocity to reach offset (reach deceleration), and grasp. The results suggest that examination of gaze patterns can inform planning strategies for control of hand shape during grasping. This information can be used as a benchmark for assessing eye-hand coordination deficits during reach-to-grasp in neurologically impaired patients such as stroke.

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Poster

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Topic: E.04. Voluntary Movements

Support: HD071978

Title: Determination of treatment algorithms for patient subgroups for post-stroke hand function rehabilitation

Authors: ***P. RAGHAVAN**[1,2], Y. LU[3], C. BAYONA[4], S. BILALOGLU[2], A. YOUSEFI[2], A. TANG[2], V. ALURU[2], A. RANGAN[3];
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Abstract: Consider a mundane task such as picking up a paper cup with coffee inside. Even this relatively simple action requires a great deal of coordination and control: one must ensure that (a) the hand posture matches the shape of the cup, (b) one doesn’t squeeze it too hard or hold it too loosely, and (c) one is able to smoothly lift it to one’s mouth. Over 85% of stroke survivors

with hemiparesis have difficulty performing these kinds of everyday tasks and – despite conventional rehabilitation – have persistent deficits in hand function that severely affect their quality of life. One of the important conclusions we have drawn from our previous experimental work is that the strategy for therapy must be targeted to the patient. That is to say, no single therapeutic strategy can be applied to all patients. This is because each patient has a unique profile of sensory and motor deficits, as well as a unique neural reserve that can be harnessed for recovery. We subject our cohort of 40 patients to a grasp and lift task under eight different learning conditions, each involving a different combination of tactile, kinesthetic and visual cues. Each patient practices the task under each of the conditions with the affected hand alone in one session, and by alternating hands in a different session. The measurements include grip and load forces, as well as several clinical tests of tactile, visual, kinesthetic and motor impairment, along with imaging data on brain lesion location and motor tract density. We will use clustering algorithms to delineate statistically significant patient subgroups which exhibit therapy-specific structure. Each of these subgroups will correspond to a 'bicluster' involving not only a specific subset of the patients, but also a specific subset of patient-traits (e.g., deficit category, clinical performance and physiological metrics). The method will pinpoint biclusters within the positively-responding patients that exhibit structure which is *not* reflected within the non-responding patients. The method can also be corrected for covariates as necessary.

Disclosures: **P. Raghavan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **Y. Lu:** None. **C. Bayona:** None. **S. Bilaloglu:** None. **A. Yousefi:** None. **A. Tang:** None. **V. Aluru:** None. **A. Rangan:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH/ NICHD grant 1R01HD071978-01A1

Title: Plasticity in cortical control signals to muscles in pianists with overuse injury with peripheral behavioral intervention

Authors: ***S. BILALOGLU**[1], S. CHAKRABARTY[2], Y. LU[3], A. YOUSEFI[1], P. RAGHAVAN[4,1];
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Abstract: Tension in neck muscles have been shown to be associated with overuse injury in pianists, but the relaxation of these muscles have not been found to be an effective treatment. To address this issue we examined cortical control of muscles by analyzing the EMG frequency spectrum in finger flexor and extensor muscles when 31 professional pianists with at least 10 years of experience played octaves; 20 of them were asymptomatic for overuse injury, whereas 11 were symptomatic. Subjects were tested under three postural conditions: (1) at baseline, (2) with relaxation of neck muscles (upper trapezius, UT), and (3) with activation of scapular stabilizers (lower trapezius, LT). EMG biofeedback was used to ensure that muscles were relaxed/ activated sufficiently. Bipolar surface electrodes were placed over flexor digitorum and extensor digitorum communis muscles. Electromyography (EMG) signals were pre-amplified and sampled through Spike 2 (Cambridge Electronic Design, Cambridge, England). Data was exported at 2000 Hz and imported into Matlab for data processing. EMG was filtered using bandpass filter (3-250 Hz, 5th order Butterworth) and 3 notch filters (notch at 60,120 and 180 Hz, Q factor 30, second order IIR). For each condition subjects played the C octave for 15 sec (7-8 times) with a pause of 2 seconds between each press; 3 such trials were recorded. The normalized power of the beta band (15-30 Hz) and the gamma band (30-55 Hz) in 70 ms bins before and after sound-onset were calculated. We found that asymptomatic pianists showed increased beta band power in their finger extensors (antagonists) during key press at baseline and with activation of the LT, but showed the opposite pattern with UT relaxation. The beta band power reflects the cortical inhibitory signal to the antagonist muscle. Symptomatic pianists, in contrast, showed decreased beta band power in the antagonist muscle at baseline and with UT relaxation, but a pattern more similar to asymptomatic pianists with LT activation.
These results suggest that when the UT is relaxed, cortical inhibition of the antagonist is reduced, leading to increased tension in these muscles. These data suggest that cortical inhibitory control is abnormal in symptomatic pianists relative to asymptomatic pianists, and points to a behavioral intervention that can alter the cortical control signal. We suggest that the targeted activation of scapular-stabilizing muscles rather than the relaxation of neck muscles may prevent overuse injury during skilled movement.

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Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

Support: MR/K01384X/1

Title: Selective and global inhibition of interneuron circuits in human motor cortex during movement preparation

Authors: *R. HANNAH, S. TREMBLAY, J. C. ROTHWELL;
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Abstract: Movement preparation has been suggested to be associated with a brief "global" inhibition of primary motor cortex (M1) excitability [1], affecting both task-relevant (responding) and task-irrelevant (non-responding) muscle representations. We asked whether this preparatory inhibition is global or selective for specific excitatory interneuron circuits in M1. We examined in 11 human participants how the excitability of two distinct circuits was affected at various stages during a warned reaction time task (WRTT), in which a warning cue appeared 500 ms prior to the imperative signal. A controllable pulse parameter transcranial magnetic stimulation (cTMS) device [2] was used to activate distinct interneuron circuits in M1 by changing the orientation of the induced current in the brain (posterior-anterior, PA; anterior-posterior, AP) and pulse width (30 - 120 μs). Motor evoked potentials (MEPs) were elicited in the first dorsal interosseous (FDI) muscle of the right hand using short AP (AP_{30}) and long PA (PA_{120}) pulses. Separate blocks of the WRTT were performed to evaluate AP_{30} and PA_{120} MEPs when responding with the right and left index fingers. Because the specificity of TMS pulses for distinct interneuron circuits is optimal at low intensities, participants maintained slight voluntary muscle contraction (5-10% maximum) throughout the WRTT to lower the threshold for stimulation. MEPs evoked by AP_{30} pulses had a longer latency than those evoked by PA_{120} (23.4 vs. 22.1; *t*-test, $P < 0.001$), confirming that different excitatory interneurons were recruited by the different combinations of pulse width and orientation. When responding with the right index, AP_{30} MEPs in the right FDI showed greater suppression than PA_{120} MEPs after the imperative stimulus (-31% v. -18%; Bonferroni corrected *t*-test, $P = 0.032$). In contrast, when responding with the left index, AP_{30} and PA_{120} MEPs in the right FDI were similarly suppressed after the imperative stimulus (-32% v. -31%; $P = 1.0$). The present data showed inhibition of MEPs during motor preparation in the responding muscle and in a muscle that is homologous and contralateral to the responding digit. The new result is that inhibition of the responding muscle was shown to be more selective for AP_{30}-sensitive interneurons, whereas the non-responding muscle showed a broader inhibition of both AP_{30}- and PA_{120}-sensitive inputs. These data point to partially independent forms of inhibition which may have different functional relevance and involve different neural networks.

References

[1] Greenhouse et al. 2015 *J Neurosci*, 35:10675-10684
[2] Peterchev et al. 2014, *J Neur Eng,* 11:056023

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Poster

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Title: Differential effects of rTMS on motor cortex excitability, interhemispheric inhibition and performance in elderly people.

Authors: M. WISCHNEWSKI[1], G. M. KOWALSKI[1], J. FREEMAN[1], S. R. BELAGAJE[1], G. HOBBS[2], *C. M. BUETEFISCH[1];
[1]Neurol., Emory Univ., Atlanta, GA; [2]West Virginia Univ., Morgantown, WV

Abstract: There are conflicting reports on the effects of low frequency repetitive transcranial magnetic stimulation (rTMS) on primary motor cortex (M1) excitability and behavior. A comprehensive study investigating effects of rTMS on excitability of right and left M1, their interaction and relationship to performance is lacking. In a sham-controlled, randomized, double-blind study, we investigated the effects of two different rTMS interventions applied to left M1 on the excitability of each M1, interhemispheric inhibition (IHI) and hand performance. Participants were 11 healthy right handed volunteers (8 F, age = 61.6 ± 1.8 years). 1Hz rTMS at 80% and 90% of resting motor threshold (MT) and 1Hz sham stimulation at 80% MT were tested. EMG activity was recorded from the extensor carpi ulnaris muscles. Stimulation effects on M1 excitability were investigated by comparing pre- and post-intervention 1) stimulus response curve (SRC, 30-80 % maximum stimulator output) measured by a Boltzmann curve with maximum MEP (M_{MAX}), growth rate (K) and inflection point (S_{50}) as parameters; 2) Short intracortical inhibition (SICI), where a conditioning stimulus (CS) of 60% or 80% of MT preceded a test stimulus (TS) of 120% of MT by an interstimulus interval (ISI) of 2 ms; 3) IHI, where a CS to one M1 preceded a TS to the other M1 by an ISI of 10 ms. Performance was measured by the participants' accuracy on a joy stick operated pointing task where a cursor was moved to targets of different sizes at random locations as quickly as possible. An ANOVA tested the effects of stimulation on SICI, IHI and accuracy of hand performance. For SRC, the fit of a difference of curves were compared to the fit of a single curve. There was a significant effect of left M1 rTMS at intensities of 80% and 90% of MT on left but not right M1 SRC with an

increased M_{MAX}, decreased K and increased S_{50} from pre- to post-intervention. Left but not right M1 SICI was decreased after rTMS at 80%. This was not seen for rTMS of 90% MT. No clear effects of rTMS were found on IHI in either direction. There was a tendency for rTMS to affect accuracy depending on the performing hand. These preliminary findings suggest that both excitatory and inhibitory circuits are modulated differently by left M1 1Hz rTMS depending on intensity. In this population of elderly subjects, the effects of rTMS on M1 excitability seems to be limited to the stimulated M1. Further, evidence for rTMS modulation of IHI was not demonstrated. This contrasts the proposed model where 1 Hz subthreshold rTMS reduces excitability of the stimulated M1 and thereby the inhibitory effect towards the non- stimulated M1. Results are consistent with reported effects of age on motor physiology and control.

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Poster

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Topic: E.04. Voluntary Movements

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National Science and Engineering Research Council (Canada)

Title: Anticipatory corticospinal control of motoneurons during self-unloading of wrist muscles: comparison with usual unloading

Authors: *L. ZHANG, A. FELDMAN;
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Abstract: When pre-loaded muscles are suddenly unloaded, the respective joint angle involuntarily moves to a new steady-state position (the unloading reflex or natural unloading). In contrast, during self-unloading produced by the subject using the contralateral hand, the joint angle remains unchanged. It has previously been shown that corticospinal influences on motoneurons of wrist muscles are maintained during natural unloading, despite changes in the joint angle and EMG activity of wrist muscles. It has also been shown that tonic corticospinal influences set the threshold position at which the wrist muscles begin to be activated. We tested the hypothesis that in contrast to natural unloading, to preserve the wrist position during self-unloading, corticospinal influences start appropriately changing the threshold position prior to

the unloading onset (anticipatory process). To test this hypothesis, we compared changes in motor-evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) applied to the wrist area in motor cortex prior to natural and self-unloading. MEPs were recorded electromyographically from four wrist muscles (flexors: FCR, FCU; extensors: ECR, ECU). Results (n=12, healthy subjects) showed that shortly before self-unloading onset, MEPs in pre-loaded flexor muscles decreased in 11 subjects and MEPs in extensors increased in 7 subjects, even though the EMG levels and the wrist position before unloading remained unchanged. In contrast, before natural unloading, MEPs in all muscles were preserved. The findings suggest that in order to prevent joint motion during self-unloading, the corticospinal system initiated a change in the subthreshold facilitation of agonist motoneurons in anticipation of unloading. The EMG and kinematic patterns of behavior in natural and self-unloading are explained based on the notion of threshold position control. It is concluded that such control has the feed-forward, anticipatory nature and is mediated by the corticospinal system.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Cortical coherence during movement.

Authors: *A. O'KEEFFE, N. POURATIAN;
Neurosci., UCLA Hlth. Syst., Los Angeles, CA

Abstract: Movement is thought to be governed by interactions among a hierarchy of cortical areas including primary motor cortex (M1), premotor cortex (PM) and the supplementary motor area (SMA). These cortical regions display movement related neural activity however it remains unknown how the interactions between movement cortices is regulated. We propose that coherent neural oscillations in the 12-35Hz band regulate inter-regional communication to facilitate the execution of cortically represented movement plans. Eight patients undergoing deep brain stimulation (DBS) for Parkinson's disease (PD) had local field potentials recorded from motor and premotor cortices during rest and cued movement. Spectral analyses show (1) low beta suppression within motor and premotor cortices during movement (2) that each subject demonstrates an increase in beta band coherence during movement and (3) that movement

related coherence peaks are correlated with peaks in the beta power spectra of premotor and motor cortices during movement. We have characterized the temporal and frequency dynamics of beta frequency spectral and coherence peaks in order to better understand the role for coherent neural oscillations in cued movement execution. An improved understanding of the means by which coherence in neural oscillations is facilitating movement could lead to development of minimally invasive cortical neurostimulation devices that induce interregional coherence to target akinetic symptoms in movement disorders.

Disclosures: **A. O'Keeffe:** None. **N. Pouratian:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 436.13/UU10

Topic: E.04. Voluntary Movements

Title: A comparison of widespread motor inhibition during movement preparation and stopping

Authors: *I. GREENHOUSE, L. CAO, R. B. IVRY;
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Abstract: Widespread inhibition is detectable in both task-relevant and task-irrelevant muscles during response preparation and during the stopping of initiated actions. Neuroanatomical models suggest a common basal ganglia-thalamic inhibitory pathway is engaged during these two phases of movement. Based on this, we predicted a positive correlation between the level of inhibition observed during stopping and the level of inhibition observed during response preparation.
Participants (n=11) used either the left index finger or right pinky finger to respond in a stop signal task (36% Stop trials) or a delayed response task (Go), resulting in a fully-crossed, two (response finger) by two (task) design, consisting of four distinct task conditions: L index Go, L index Stop, R pinky Go, and R pinky Stop. The four conditions were tested in separate blocks, with the imperative always preceded by a preparatory delay period. Transcranial magnetic stimulation (TMS) was applied over right primary motor cortex to elicit motor evoked potentials (MEPs) from the left first dorsal interosseous (FDI) muscle when that muscle was relevant (L index) or irrelevant (R pinky) to the tasks. MEPs were measured 800 ms into the 900 ms preparatory delay interval on a subset of trials during both tasks. On separate trials of the R pinky Stop condition, MEPs were also measured 150 ms after the imperative (Go trials) and 200 ms after the stop signal (Stop trials). We did not apply TMS after the stop signal in the L index condition because the MEPs would be elevated due to ongoing movement implementation.

The left FDI was significantly inhibited during the preparatory delay relative to an inter-trial baseline in all conditions (p's < .05) except the L index Stop condition (p = .70). The left FDI was significantly more inhibited when preparing R pinky responses than L index responses (p = .001). There was no main effect of task and no interaction. Left FDI MEPs measured during successful and failed stopping in the R pinky Stop task were suppressed relative to baseline (both p < .005) and relative to MEPs measured after the imperative on go trials (both p < .05). The level of preparatory inhibition in the R pinky Stop condition was significantly correlated with the level of inhibition during successful stopping (R = .86, p < .005) and during failed stopping (R = .71, p < .05). However, MEPs measured during the delay period were not correlated between task conditions (all p > .06). These results are consistent with the hypothesis that a shared inhibitory mechanism with a widespread effect on the motor system is engaged during the preparation of responses and during stopping.

Disclosures: I. Greenhouse: None. **L. Cao:** None. **R.B. Ivry:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 436.14/UU11

Topic: E.04. Voluntary Movements

Support: James McDonnel Foundation 220020220

Title: Abnormal interhemispheric interactions are present in the chronic but not in the acute or subacute post-stroke periods

Authors: *J. XU[1], M. BRANSCHEIDT[3,2], H. SCHAMBRA[4], G. LIUZZI[3], L. STEINER[3], N. KIM[1], T. KITAGO[4], A. LUFT[3], J. W. KRAKAUER[1], P. A. CELNIK[2];
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Abstract: The exact mechanisms of motor recovery following stroke remain poorly understood. Previously Murase et al. (2004) described in 12 chronic stroke patients the presence of persistent interhemispheric inhibition (IHI) from the healthy to the ispilesional motor cortex in the context of movement. This finding was associated with muscle strength where patients with lowest MRC have less release of IHI, hence more inhibition at movement onset. This classic study has been highly influential in the neurorehabilitation field leading to the test of many interventions geared towards rebalancing this abnormal IHI. However, to date it is unclear whether unbalanced IHI is present early after stroke, how it evolves over time, and if it has any predictive value for motor

recovery.
Here we studied over one year the evolution of movement-related interhemispheric inhibition (IHI) in patients with first-time acute onset unilateral, cortical or subcortical ischemic stroke leading to a motor deficit of the upper limb (N=22) and healthy age-matched controls (N=11). Participants were tested at 5 different time points after stroke (week 1, 4, 12, 24, and 52). At each session, participants performed a simple reaction time task, in which they made voluntary abduction movements with the index finger of their paretic hand. Paired-pulse TMS was applied at four different timings during movement preparation, with the conditioning stimulus applied to the M1 of the healthy hemisphere and the test stimulus over the lesioned hemisphere (contralateral to the paretic hand). Healthy controls always performed the task with their right hand, while the conditioning pulse was applied to the right M1 and test pulse to the left M1 (contralateral to the moving hand).
Contrary to previous believes, we found that in the acute to subacute stages (weeks 1, 4, and 12) patients showed release of pre-movement IHI similar to healthy controls ($p = 0.59$). Interestingly, the amount of release of IHI early after stroke correlated with hand strength at chronic stages (mean of week 24-52; $r = 0.78$, $p = 0.0005$). Noteworthy, in the chronic phase (weeks 24 and 52) patients have less release of IHI compared to healthy controls ($p = 0.006$), consistent with Murase et al. report. Our findings suggest that abnormal interhemispheric interactions in chronic stroke patients could be the consequence of a maladaptive process not present early after stroke. In addition, early pre-movement IHI might have predictive value for hand strength recovery.

Disclosures: **J. Xu:** None. **M. Branscheidt:** None. **H. Schambra:** None. **G. Liuzzi:** None. **L. Steiner:** None. **N. Kim:** None. **T. Kitago:** None. **A. Luft:** None. **J.W. Krakauer:** None. **P.A. Celnik:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

Support: Indiana University Faculty Research Support Program (FRSP)

Title: Somatotopic specificity of motor cortex plasticity in response to visuo-proprioceptive realignment

Authors: ***J. L. MIRDAMADI**[1], A. K. LYNCH[1], Y. LIU[1,2], H. J. BLOCK[1,2];
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Abstract: Goal-directed reaching requires integration of visual and proprioceptive information about the hand. In general, these estimates are combined and weighted in a statistically optimal manner. When we encounter a perturbation that causes a misalignment between visual and proprioceptive estimates, movements initially will be inaccurate. The brain can resolve this issue through changes in multisensory perception including weighting and realignment, or through motor adaptation. Although these processes have traditionally been studied in isolation, recent behavioral and neurophysiological studies have shown that motor adaptation influences proprioceptive realignment. Whether this link between perceptual and motor systems is reciprocal is unknown. Here, we investigate the effect of visuo-proprioceptive realignment on motor cortex (M1) excitability. Seated at a reflected rear projection touchscreen apparatus, subjects performed right hand movements to proprioceptive targets (left index finger), visual targets (white box), or both together. In one of two sessions, no misalignment was introduced. In the other session, a 70 mm misalignment was induced gradually by shifting the white box away from the left index finger. Before and after the behavioral task, single pulses of transcranial magnetic stimulation (TMS) were delivered over the hand region of M1 contralateral to the left index finger at rest. To determine whether any effects on M1 excitability are somatotopically specific, we computed input-output (IO) curves for both first dorsal interosseous (FDI) and abductor digiti minimi (ADM). Data were fitted to a Boltzmann sigmoid function. Preliminary data (n = 5) indicate that individual M1 excitability changes may be related to multisensory integration. FDI IO slope increased to a greater extent in individuals who relied more on proprioception than vision in the misaligned session, but not veridical session. According to the minimum variance model, the lower weighted (least trusted) modality is expected to realign more. Therefore, a lower weight of vision would be associated with more visual realignment and less proprioceptive realignment. These initial results are in line with our previous findings on correlations between IO slope change and realignment, and further data will clarify whether this is the case in ADM as well as FDI. Overall, the data suggest that the effect of misalignment on motor cortex excitability is a function of how individuals integrate multisensory information. This has important implications for current models of multisensory integration and motor control.

Disclosures: **J.L. Mirdamadi:** None. **A.K. Lynch:** None. **Y. Liu:** None. **H.J. Block:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

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Program#/Poster#: 436.16/UU13

Topic: E.04. Voluntary Movements

Title: Investigating the temporal aspects of action observation: evidence from soccer players

Authors: ***M. BOVE**, L. PEDULLÀ, E. GERVASONI, A. BISIO, M. BIGGIO, L. AVANZINO;
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Abstract: In everyday life we are constantly subjected to visual stimuli constituted by other people actions, producing a wide sequence of neural mechanisms in our brain. One of the contexts where action observation (AO) occurs is sport. A particular aspect of this phenomenon is given by the spectators' reaction following temporal errors committed by the athletes. It is now widely accepted that AO elicits an increase of the observer's corticospinal excitability and it is assumed that the perceived actions are matched to a representation of the observer's own actions depending on his/her previous motor experience. To date, the effect of an observed delayed action on the observer's motor system is still unknown. The aim of the present study was to investigate how different spatio-temporal patterns in the execution of a soccer action can affect corticospinal excitability in soccer experts (Soccer group: N=16) and in novices (Novice group: N=10). To this aim subjects were asked to watch two videos representing the same action: a correct video, where a pass was performed with the correct timing, and a delayed video, where the pass was delayed with respect to the action development. Corticospinal excitability during AO at different time points was assessed by means of transcranial magnetic stimulation for the right tibialis anterior, a muscle involved in the soccer pass movement. Considering the correct video, in the Soccer group, corticospinal excitability recorded at the moment in which the ball was passed was significantly higher than that recorded after the pass. On the contrary, in the delayed video, the Soccer group showed a significantly higher corticospinal excitability about 800 ms before the ball was passed than that recorded at the beginning or at the end of the action, i.e., when the pass was really performed. No statistically significant differences were reported for the Novice group in the different timing points for both videos. These results confirm that the observer’s motor experience strongly influences corticospinal excitability during the observation of a well known action. Further, motor experience, likely through a mechanism of temporal prediction, affects the activity of the neural system underpinning action observation inducing an increase of the corticospinal excitability when the action is expected and not when it is actually observed.

Disclosures: **M. Bove:** None. **L. Pedullà:** None. **E. Gervasoni:** None. **A. Bisio:** None. **M. Biggio:** None. **L. Avanzino:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

Title: The competitive effect of alternative movements with different probabilities on EEG during preparation period

Authors: *A. FUJIKAWA, Y. MATSUMOTO, T. URAKAWA, O. ARAKI;
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Abstract: Before a human or a monkey begins an action, neural activities relevant to preparation for the movement is observed. Neurophysiological studies in monkeys have suggested that the premotor and motor cortices simultaneously prepare for multiple movements, when multiple candidates of movements are given. Electroencephalogram (EEG) studies in humans reported that amplitude of Lateralized Readiness Potential (LRP) related to the movement preparation decreased as the number of possible movements increased (Praamstra et al. 2009). The LRP was interpreted to originate from competition among different preparation activities. Since increasing the number of the movement candidates corresponds to decreasing probability for each movement candidate, there remains a possibility that the amplitude of LRP reflects the probability for each movement, irrespective of competition. In fact, it has been reported, in Go/No-go task, that the higher the probability to move is, the larger the amplitude of LRP becomes. Moreover, the amplitude of Contingent Negative Variation (CNV) is also larger when the prior probability to move is higher. Thus, we fixed the probability of movement to one direction (80%) to determine whether EEG activity reflects the effect of competitive interaction between probable movements rather than the probability itself. We designed two conditions: (1) to move to another direction (20%), and (2) no movement (0%). Under the constant probability, we can compare the results of competitive and no competitive conditions. In this task, we first presented 1 or 2 spatial cues whose positions indicated the potential target's location(s). The thickness of lines in the spatial cue implied go probability, and subjects were asked to prepare for the movement based on prior probability. A move cue was then presented and the cue indicated whether or not participants had to move their hands to the target. When the cue indicated 'move', the cue also indicated the location of the actual target. Results showed that CNV amplitude in the competition condition was larger than that in the no competition condition at 800-900 ms after the spatial cue's onset ($p<0.05$). In contrast, there was no significant difference in LRP amplitude between the two conditions. These results indicate that the effect of competitive interaction is reflected by CNV rather than LRP during the period of movement preparation. In addition, the significant difference in CNV amplitude appeared at 300-400ms before the move cue. Thus, the

neural processing related to competition is considered to be evoked in the early stage of movement preparation.

Disclosures: **A. Fujikawa:** None. **Y. Matsumoto:** None. **T. Urakawa:** None. **O. Araki:** None.

Poster

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Topic: E.04. Voluntary Movements

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Title: Electroencephalography based analysis of hemispheric activation asymmetry for goal- and non goal-oriented movements with virtual mirror feedback

Authors: ***M. ROHAFZA**[1], M. YAROSSI[2], E. TUNIK[3], S. ADAMOVICH[1];
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Abstract: Therapy for stroke often uses repetitive task practice, which is difficult to perform for patients with severe impairment. Mirror feedback (MF) therapy that uses motion of the less affected limb is potentially beneficial for recovery and can recruit the lesioned hemisphere. However, the rehabilitation outcomes vary and the neural mechanisms underlying MF are poorly understood. One contribution to the conflicting results is wide variability in the type of sensorimotor motor task that is combined with MF. We investigated the interaction of goal (target, no target) and visual feedback (veridical feedback (VF), MF) in a 2x2 factorial design. We used electroencephalography (EEG) to investigate hemispheric activation asymmetry in a timing specific manner to dissociate processes related to movement planning and execution. Eighteen healthy subjects (age: 22±3) performed fast right index finger flexion. Movements were recorded with a data glove to actuate, in real time, virtual hands shown on a display that was placed above their hands. Subjects completed four sessions with MF with a target (MG+), MF without a target (MG-), VF with a target (VG+), and VF without a target (VG-). Hemispheric asymmetry, the difference (sum squared error, SSE) in event related de-synchronization (ERD) in the beta band (15-25Hz) between motor cortices (electrodes C3 and C4), was quantified over 4s epochs at baseline (before cue presentation), planning (between cue presentation and

movement), movement execution, and at post (after movement completion). The main effect of visual feedback on asymmetry index was significant during both preparation and execution. The goal x visual feedback interaction was significant during planning only. Although there was no significant main effect of goal during planning or execution, the presence of a goal in the veridical condition was associated with increased lateralization of activity during planning. This effect may be due to inhibitory interhemispheric interactions that are in place to prevent mirror movements. In the presence of a goal, MF significantly decreased hemispheric asymmetry, compared to VF, with MG+ having the lowest asymmetry index during both planning and execution epochs. Together, these results indicate that mirror feedback may cause decreased asymmetry in sensorimotor cortices, with a distinct effect of goal on movement planning. Our study sheds light on the timing of interhemispheric interactions during MF in healthy individuals. The results will guide future investigations into these mechanisms in stroke populations and the application of mirror therapy and brain stimulation protocols to optimize therapy outcomes.

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Poster

436. Cortical Planning and Execution: Human Physiology

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Program#/Poster#: 436.19/VV2

Topic: E.04. Voluntary Movements

Title: The influence of cerebellar transcranial direct current stimulation on motor skill acquisition and learning in a throwing task

Authors: ***B. J. POSTON**[1], L. L. ALBUQUERQUE[1], A. K. JACKSON[1], K. M. FISCHER[1], M. A. GUADAGNOLI[1], Z. A. RILEY[2];
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Abstract: Cerebellar transcranial direct current stimulation (c-tDCS) is a non-invasive brain stimulation technique that has been shown to increase motor performance in simple tasks such as planar arm movements. However, the ability of c-tDCS to enhance motor performance in a complex, goal-directed task involving whole body coordination is unknown. The purpose was to determine the influence of c-tDCS on motor skill acquisition and learning in a throwing task in young adults. The study was a double-blind, sham-controlled, between-subjects experimental design. Twenty-four young adults were allocated to either a c-tDCS group or a SHAM stimulation group. Each subject participated in a practice session followed by an experimental session, which was completed 24 hours later. In the practice session, subjects performed 10 trials

of a throwing task in a baseline testing block, followed by 6 practice blocks of 10 trials, and a post-testing block of 10 trials that was performed five minutes after the last practice block. In the retention session, subjects performed the throwing task for a single block of 10 trials. The throwing task involved accurately throwing tennis balls puts to a small target placed on a concrete wall 6 meters away from the subjects. Anodal c-tDCS or SHAM stimulation was applied during the practice blocks at a current strength of 2mA for 25 minutes. For c-TDS, the anode was place 3cm lateral to the inion overlying the cerebellar hemisphere ipsilateral to the throwing arm, whereas the cathode was placed over the ipsilateral buccinator muscle. SHAM stimulation was applied in the same manner according to established blinding procedures in which the current was ramped up and down over a period of 60 seconds. The dependent measures of interest for the throwing task were endpoint error and endpoint variance. The endpoint error was quantified as the absolute distance of the final endpoint of each throw relative to the target, whereas the endpoint variance was quantified as the sum of the variances of the *x* and *y* endpoints for each trial block. For the testing blocks, the endpoint error and endpoint variance were similar at baseline between the two groups, but were lower in the c-tDCS group compared with the SHAM group for the post-test block and the retention testing block. However, the rate of decline in endpoint error and endpoint variance was similar for the two groups during the actual practice blocks. The findings indicate that a single application of c-tDCS applied during practice of a complex throwing task increases endpoint accuracy and reduces endpoint variability in young adults when measured in retention tests performed 5 minutes and 24 hours after cessation of practice.

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Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

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Title: Diverse patterns of movement related potentials after EEG BMI intervention in severe chronic stroke patients

Authors: *O. YILMAZ[1,2], D. BROETZ[2], W. CHO[2], E. GARCIA COSSIO[2], G. LIBERATI[2,3], F. BRASIL[2], M. ROCHA CURADO[2], N. BIRBAUMER[2,4], A. RAMOS MURGUIALDAY[2,5]; [1]psychology, Bahcesehir Univ., istanbul, Turkey; [2]Inst. for Med. Psychology and Behavioural Neurobio., Univ. of Tuebingen, Tuebingen, Germany; [3]Inst. of Neuroscience, Univ. catholique de Louvain, Brussels, Belgium; [4]Ospedale San-Camillo, Venezia-Lido, Italy; [5]Neurotechnology Laboratory, TECNALIA Hlth., San Sebastian, Spain

Abstract: Movement related cortical potentials (MRCPs) proposed as reliable and immediate indicators of cortical reorganizations in motor learning. It has been reported that decrease in amplitude and later onset of MRCPs reflect less mental effort and shorter planning time during a motor task. In this study MRCPs preceding hand movements in severe chronic stroke patients were investigated using an EEG paradigm (patients performed hand open and close for paretic and healthy hand) before and after a one-month online EEG Brain Machine Interface neurorehabilitation intervention coupled with physiotherapy. 17 severely impaired (no residual finger extension) chronic stoke patients were participated in the study. In the group of patients who received contingent feedback MRCP peak amplitude for paretic hand movements was significantly reduced over Cz and MRCP onset for paretic hand movements was significantly later over central regions in the post measurements comparing pre results. No significant changes measured in the sham-feedback group and healthy hand movements between pre-post measures. Our results suggest that our patients needed less mental effort and shorter planning time after intervention. We demonstrated for the first time significant MRCP changes after a neurorehabilitation intervention (BMI and physiotherapy) in severe chronic stroke patients.

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Poster

436. Cortical Planning and Execution: Human Physiology

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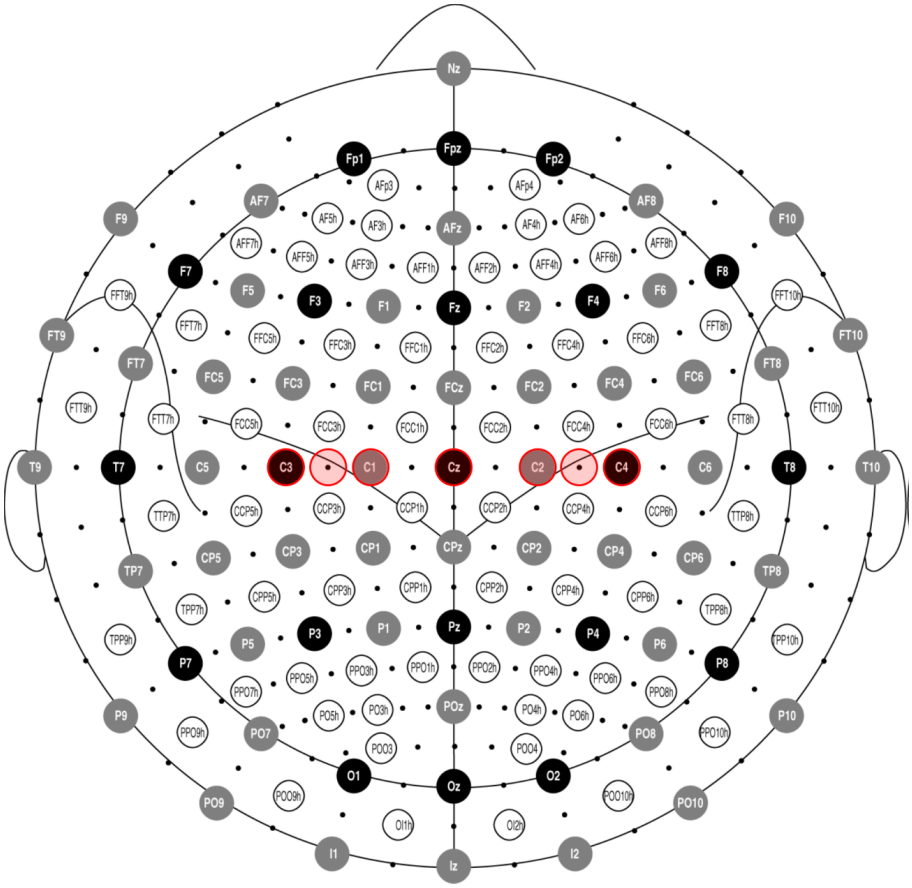
Topic: E.04. Voluntary Movements

Support: TUBITAK 2232 grant (115C091)

Title: Minimizing the required number of surface EEG electrodes with focused positioning

Authors: *M. B. BAYRAM[1,2], H. ARGUNSAH BAYRAM[1,2];
[1]Dept. of Med. Engin., Acibadem Univ., Istanbul, Turkey; [2]Human Performance and Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: Introduction: This study examines applying real-time PSD analysis to surface EEG signals which acquired by the minimum number of electrodes. While recording high-density EEG signals, most of the "unneeded" data are discarded in the pre-processing stage. Additionally, it is known that EEG frequency power (EEG_{FP}) of some EEG rhythms has a relationship with the voluntary motor activities, which could further state that EEG_{FP} controls the muscle contraction level: the higher the EEG_{FP}, the more the muscle contraction. The current study tested a hypothesis that, by implementing this relationship and novel feature extraction techniques, using only 7 or less electrodes would compare to 32-256 channel EEG recordings' findings. **Methods:** In Phase One, 2 healthy young (20.23 ± 1.23 years) individuals performed ~3s lasting isometric maximum voluntary contractions (MVC) of the non-dominant arm elbow flexion (EF) for 40 times, with 8s rests in between. Then, subjects were asked to repeat the procedure with their dominant arm, for 40 times. After a 10-minute rest, in Phase Two, they were asked to imagine the EF by following the same protocol. Meanwhile, 7 scalp EEG signals were recorded during the motor and imaginary tasks imaginary MVC contractions. **Results:** Our preliminary findings showed that Beta-band EEG_{FP} exhibited significant positive correlation with the EF force for both physical and imaginary contractions for the contralateral side; meanwhile for imaginary MVC, Alpha-band EEG_{FP} had also significant negative correlation. Additionally, for this preliminary study with limited number of participants, removing some of the electrodes' data caused inconsistencies within the findings. **Conclusions:** EEG_{FP} of some EEG rhythms has a direct relationship with physical and imaginary voluntary motor activities. Minimizing the number of electrodes by focusing the placement that can gather this relationship data, would lead to the faster processing. These findings may be used as a marker for a faster and more reliable Brain-Computer Interfaces, for both research and clinical.



The 10-5 electrode system has been suggested by Oostenveld et al (1). Based on this montage, Cz (as reference), C1, C2, C3, C4, C3h and C4h electrodes have been used for this study (indicated in red). (Oostenveld R, Praamstra P. The five percent electrode system for high-resolution EEG and ERP measurements. Clin Neurophysiol. 2001;112(4):713-719.)

Disclosures: **M.B. Bayram:** None. **H. Argunsah Bayram:** None.

Poster

436. Cortical Planning and Execution: Human Physiology

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Topic: E.04. Voluntary Movements

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Title: Visual perception threshold is inversely correlated with moment-to-moment changes in corticomuscular coherence during tonic isometric voluntary ankle dorsiflexion in humans

Authors: *R. MATSUYA[1], J. USHIYAMA[2,4], S. KASUGA[3], J. USHIBA[3];
[1]Keio Univ. Grad. Sch. of Sci. and Technol., Kanagawa, Japan; [2]Fac. of Envrn. and Information Studies, [3]Fac. of science and Technol., Keio Univ., Kanagawa, Japan; [4]Departiment of Rehabil. Med., Keio Univ. school of Med., Tokyo, Japan

Abstract: BACKGROUND: Oscillatory activity of the sensorimotor cortex measured by electroencephalogram (EEG) shows coherence with electromyogram (EMG) activity in a contralateral limb muscle within the 15-35 Hz frequency band (β-band) during tonic isometric voluntary contraction. Previous studies have reported that the magnitude of the corticomuscular coherence (CMC) can be changed by various factors including motor learning, attention and cognitive effort. From these findings we speculate that visuomotor feedback gain is associated to the CMC modulation caused by the learning or cognitive control. Indeed, the visuomotor feedback gain is known to be enhanced during the learning process. Recently, we reported that CMC changed from moment to moment even during tonic isometric contraction of a same motor level, and also this change affected the motor performance. Here, we aimed to investigate whether visual perception threshold is altered in association with the moment-to-moment changes in the magnitude of CMC during the serial motor task, to characterize the visuomotor feedback gain in the CMC modulation. METHODS: We recruited nine healthy participants with significant CMC between EEG and EMG measured from the tibialis anterior muscle. The participants performed tonic isometric voluntary ankle dorsiflexion and adjusted their contraction levels by reference to a cursor displayed on a monitor in front of participants. The participants were instructed to push a button by their right thumb immediately when they perceived cursor jumps. We set 10 types of cursor jumps with different jump sizes based on force SDs of each participants (i.e., 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 SD), and total 640 jumps were produced at random timings and in random orders. We divided the 640 trials into two groups (GD+ or GD-) including 200 trials depending on the magnitude of EMG β-band grouped discharge 1.5 s before timings of each jump occurrence. RESULTS: The magnitude of CMC in GD+ was significantly greater than that in GD- ($p < 0.001$). Perception thresholds calculated from the psychophysical curve between the cursor jump size and percentage of perception for each group demonstrated that the perception threshold of GD+ was significantly smaller than that of GD- ($p = 0.036$). CONCLUSION: This result suggests that the visuomotor feedback gain is upregulated with increased CMC.

Disclosures: **R. Matsuya:** None. **J. Ushiyama:** None. **S. Kasuga:** None. **J. Ushiba:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: ERC ParietalAction

Title: The mirror neuron system discriminates action exemplar: a human fMRI study

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Abstract: To understand which regions of human action observation network discriminate action's exemplars, we conducted an fMRI experiment in which the subjects, after watched videos showing a female ormale actor executing a manipulative action (grasping or pushing) toward an object (red cube or blue ball), have to perform three different 2-alternative forced choice tasks (2AFC) regarding to action, colour of the object or gender of the agent. To avoid lateralisation bias, the position of the actor was flipped in half of the blocks. 18 right-handed subjects were scanned in a single fMRI session including 8 experimental runs in which the three discrimination conditions and the fixation one were repeated twice. A fixation point was put in the centre of the screen close to the starting position of the hand. To check the steady fixation monocular eye-tracker monitored saccades for all the duration of the runs. Eye data results showed that subjects fixation well (average: 10.2 saccades per minute, SD=2.9); ANOVA confirmed that the number of saccades did not differ across conditions ($F_{3,12} = 0.3$ $p= 0.9$). The behavioural results showed the subjects performed discriminate very well in all of the presented tasks (av % of corrected responses for actions: 92%; gender: 95 %; colour: 96%; without difference between conditions $F_{3,12}= 0.5$ $p>0.3$). Conjunction analysis tests with inclusive masking revealed the regions activated more by one of the three discrimination tasks compared to the two others. Bilateral anterior intraparietal (phAIP) and left precentral sulcus (PCS) were more activated by action discrimination task (left phAIP: -42 -46 42 $t=5.8$ $p<0.05$ FEW corr; right phAIP: 36 -50 42 $t=6.3$ $p<0.03$ FEW corr; PCS: -42 -4 46 $t=5.9$ $p<0.05$ FEW corr); left middle temporal gyrus (MTG), left PGa-PGp and right fusiform gyrus (FG) were found specific for gender discrimination (MTG: -44 -68 0; $t=5.9$ FEW corr; PGa-PGp -38 -76 36 $t=6.1$ $p<0.04$ FEW corr; right FG: 28 -40 -20 $t=6.1$ $p<0.04$ FEW corr) while no specific region for colour task was found. This preliminary data highlighted the role of mirror neuron system (MNS) in discriminating between action exemplars. The activation of left MTG overlapping with extrastriate body area (EBA, Downing et al 2001) and which one in right FG cluster, set just posterior to fusiform body area (FBA, Peelen et al 2005), show that feature attention drawing to the gender improved the processing of region known to be involved to the processing of

observing body parts; this could mean that the arm and hand of actors were the cues used to discriminated between the two genders.

Disclosures: **S. Ferri:** None. **A. Platonov:** None. **G.A. Orban:** None.

Poster

437. Cortical Planning and Execution: MRI

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Topic: E.04. Voluntary Movements

Support: NSF BCS-1455629

James S. McDonnell Foundation 22002078

James S. McDonnell Foundation 220020293

Title: Neural correlates of performance time on a tool-use task in chimpanzees

Authors: ***L. D. REYES**[1], S. BIANCHI[3], W. D. HOPKINS[4,5], C. C. SHERWOOD[2]; [1]Lab. for Evolutionary Neurosci., [2]Dept. of Anthrop., George Washington Univ., Washington, DC; [3]Dept. of Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [5]Developmental and Cognitive Neurosci., Yerkes Natl. Primate Res. Ctr., Atlanta, GA

Abstract: The manufacture and use of complex tools is a distinctive human behavior and is associated with a left-lateralized tool circuit that includes a diverse group of cortical areas. Specific white matter tracts have been associated with the tool-use circuit in humans including the superior longitudinal fasciculus (SLF), inferior fronto-occipital fasciculus (IFOF), anterior thalamic radiation (ATR), and uncinate fasciculus (UF). This study investigated the evolution of these white matter tracts in tool-use by assessing differences in white matter structure associated with performance time on a tool-use task in chimpanzees. To this end, we used diffusion tensor imaging (DTI) scans to perform tract-based spatial statistics (TBSS) of fractional anisotropy (FA) and mean diffusivity (MD) in a sample of 44 chimpanzees. The task used to measure tool-use performance consisted of a simulated termite fishing task and performance time was measured as the average time over a number of bouts (≥50). Since sex-based differences in tool-use proficiency have been observed in both wild and captive chimpanzees, we also accounted for effects of sex, age, and brain volume in the analyses. The TBSS analysis modeled the effects of an interaction between sex and tool-use by comparing the relationship between performance time on the task and both FA and MD between males and females. A linear model was also used to

assess the overall effect of tool-use on both FA and MD. Results showed a medium effect size (Pearson's $r > 0.3$) of the interaction in both FA and MD in the SLF, ATR, corticospinal tract, anterior commissure, and corpus callosum, although the results did not reach statistical significance ($p > 0.05$). The overall effect of tool-use performance time also showed a medium effect size for both FA and MD, but was not statistically significant ($r > 0.3$; $p > 0.05$). Faster performance time was associated with greater FA in the SLF and corpus callosum, and lower FA in the ATR. Faster performance time was also associated with greater MD in the ATR, IFOF, SLF, and cerebellum. Although the human circuit is left-lateralized, there was no lateralization detected with the TBSS analysis in chimpanzees, as many of the clusters appeared bilaterally. Tool-use performance time appears to have an effect on the ATR, SLF, and IFOF, tracts that have been associated with the human tool-use circuit. These results suggest that similar tracts in the chimpanzee and human brain may be involved in tool-related processing.

Disclosures: **L.D. Reyes:** None. **S. Bianchi:** None. **W.D. Hopkins:** None. **C.C. Sherwood:** None.

Poster

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Program#/Poster#: 437.02/VV8

Topic: E.04. Voluntary Movements

Title: Task-dependent changes in alpha-band power during preparation for sensory-motor action

Authors: ***Y. MATSUMOTO**, A. FUJIKAWA, T. URAKAWA, O. ARAKI;
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Abstract: Previous electroencephalography (EEG) studies reported motor-preparation-related potentials and neural oscillations. The neural dynamics before movement depends on how to response to the stimulus (Shenoy et al., 2013). In the sensory-motor process, therefore, visual information such as position and shape of objects may influence the movement preparation. Actually, it has been reported that the stimulus difference, for example between position and meaning of figures, affects the brain activity for anticipation as well as behavioral and EEG data before and after the stimulus onset (Zoccatelli et al., 2010, Molin et al., 2013, Strack et al., 2013). Here, we hypothesize that the corresponding neural networks prepare for a sensory-motor action before the movement starts. The purpose of our study is to verify whether different brain areas are activated when humans prepare for movement in different sensory-motor tasks. We presented an integer (1-9, except 5) as a target cue above or below the fixation point. There were two conditions: (1) a subject should push the button (upper or lower) based on the position of

number (position-button: upper-upper & lower-lower, and vice versa), (2) which button to be pushed depends on the parity of the number (position-button: even-upper & odd-lower, and vice versa). We analyzed EEG during the delayed period after the task condition is indicated. In this study, we focus on alpha-band power during preparing the movement because it is known to be related to cortical inhibition: the power tends to decrease in task-related areas, while it tends to increases unrelated areas. As a result, the reaction time was longer in the parity condition than in the position condition. The alpha-band power around the motor area (electrode position C3) for parity discrimination was larger than that for position during 400 ms before a target cue was presented. These results show that the cortical activity around the motor area is inhibited strongly during preparing for the movement related to the parity discrimination. Accordingly, the result suggests that different neural networks have already been activated before movement onset depending on the anticipated sensory-motor process.

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Poster

437. Cortical Planning and Execution: MRI

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Program#/Poster#: 437.03/VV9

Topic: E.04. Voluntary Movements

Support: American Legion Brain Sciences Chair

Brain and Genomics Fund, University of Minnesota Foundation

Title: Functional motor cortical connectivity in twins and non-related individuals

Authors: *J. E. JOSEPH, P. CHRISTOVA, A. GEORGOPOULOS;
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Abstract: Introduction: We analyzed fMRI and motor behavioral data from the Human Connectome Project (HCP; www.humanconnectome.org) to explore associations between twin pairs of participants, and between randomly matched participants. Specifically, we tested the hypothesis that neural and behavioral measures would be significantly correlated in twins but not in unrelated people. In addition, we sought to estimate the contribution of the twin status to the possible associations between twins using the coefficient of determination (r^2 x 100) as an estimate of the percent of variance explained.
Methods**:** We analyzed two groups of participants**:** twins (N = 338 participants; 169 twin pairs) and non-related participants (N = 516; 258 randomly matched pairs). Resting-state, HCP fMRI

data consisted of time series of 1200 volumes per vertex, acquired with 2x2x2 mm resolution and 720 ms repetition time. These times series were prewhitened using a (15,1,1) ARIMA model (Christova, P. et al. J. Neural Eng. 8: 046025, 2011) and their innovations cross-correlated with varying lags. For left and right motor cortices we computed all possible pairwise cross correlation functions (CCF; ~2 million CCFs/motor cortex). For each CCF we located the highest absolute CCmax and noted its value and sign. We then constructed frequency distributions of the signed CCmax (CC) for each individual motor cortex to obtain the relative frequency of occurrence of negative and positive CCs. On the average, there were 30% negative and 70% positive CCs. Across all participants, the percentage of negative CCs (overall "inhibitory drive") ranged from 8% to 46%. Finally, motor behavior was assessed by calculating age-adjusted scores for dexterity, grip strength, gait speed and endurance of each participant. Results: First, we found that the test scores for the four motor behavioral tests above were significantly correlated in twins but not for non-related participants. Second, the inhibitory drives for the left and right hemispheres, and their mean, were significantly correlated in twins [r (mean inhibition) = 0.245 (P = 0.001, N = 169); r (left) = 0.233 (P = 0.002); r (right) = 0.251 (P = 0.001)] but not in non-related participants (no significant correlations for all three measures). Conclusions: The overall inhibitory drive and the motor capacities were significantly correlated between twins but not between randomly paired unrelated participants. With respect to inhibitory drive, the percent of variance explained, based on the correlation coefficients above, amounted to an average of 6% in twins, an approximate estimate of the contribution of similar genetic makeup to functional motor cortical connectivity.

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Poster

437. Cortical Planning and Execution: MRI

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Topic: E.04. Voluntary Movements

Support: University of Cologne Emerging Groups Initiative (CONNECT group) implemented into the Institutional Strategy of the University of Cologne and the German Excellence Initiative

Title: Motor network dynamics when coordinating bimanual actions

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Abstract: Even simple hand movements involve the coordinated interactions of distributed neural populations. However, these interactions can be modulated by whether a hand is moved in isolation or in parallel with the other hand's actions. Due to the diversity of bimanual actions, the mechanisms governing network modulations in bimanual contexts remain challenging to identify. Here we assessed whether the demands of producing additional movements or of inhibiting them in a bimanual context produce dissociable effects on the network dynamics and behavioral properties of a hand's actions. FMRI and Dynamic Causal Modeling (DCM) were used to this end.

In our task, right-handed participants (*N*=23*,* mean age = 27y*)* pressed buttons synchronized with periodic visual stimuli (2.2Hz) over 28 second blocks. In each block, movements were made either with (i) the right hand; (ii) left hand; (iv) both hands together; or (iii) both hands alternating. Periodic cues induced alternating Go states (having 4 movements) and NoGo states of equal duration. With this structure, the unilateral and Alternating conditions had matched Go states requiring movements with only one hand, while the unilateral and Together conditions had matched NoGo states where all movements were halted irrespective of hand.

The unilateral and Together conditions (matched NoGo states) showed strong behavioral similarities. The mean response time (RT) for both hands decreased from the first to the fourth stimulus in both the unilateral and the Together condition. However, this RT pattern was absent in the Alternating condition. Furthermore, individual RTs in the unilateral and the Together conditions were highly correlated implicating a common mechanism. Connectivity was assessed in a bilateral cortical network consisting of M1, PMd, SMA and IPS. During unilateral movements, bilateral SMA was positively coupled to contralateral M1 and negatively coupled to ipsilateral M1. This connectivity pattern was substantially modified in both bimanual conditions. Despite the shared Go state in the Alternating condition, bilateral SMA had a positive coupling to both contra- and ipsilateral M1. This pattern was shared by the Together condition with a critical difference. Right M1 was modulated to a greater extent by left SMA in the Together condition and by left PMd in the Alternating condition.

In summary, our findings suggest that, in a bimanual context, the demand for selective inhibition modulates each hand's action to a greater extent than the added movement demands. The differential roles of SMA and PMd might be linked to these inhibitory differences in producing coordinated bimanual actions.

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Poster

437. Cortical Planning and Execution: MRI

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Program#/Poster#: 437.05/VV11

Topic: E.04. Voluntary Movements

Support: ARL CaNCTA

ORAU

Title: Increased locomotor demand is associated with decreased cortical alpha power

Authors: *J. R. LUKOS[1], J. C. BRADFORD[2], D. P. FERRIS[3];
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Abstract: The effect of performing simultaneous cognitive and motor tasks can affect overall task performance compared to when each task is done in isolation. However, this relationship can depend on the intensity of tasks performed. The goal of this study was to better understand the underlying neural processes associated with locomotion under variable cognitive and physical loads. We measured EEG while participants (N=18) walked on a treadmill with an empty backpack (unloaded condition) or a heavy backpack (40% body weight, loaded condition) at a 1 m/s for 1 hour. While walking, subjects also performed a visual oddball paradigm where 12% of displayed images were targets that required participants to respond with a button press. We hypothesized that there would be increased engagement in locomotor related cortical areas while walking with the heavy backpack and decreased engagement in locomotor related areas during dual tasking with the visual oddball task. To test this, we processed EEG data by removing bad channels, applying independent component analysis, and back-projecting cortical independent component weights to channel data. We tested the differences in the frequency spectrum during loaded and unloaded conditions while either walking in isolation or performing the cognitive task. We computed log power spectra for each channel using a Wilcoxon (rank-sum) test to evaluate mean power differences between conditions within a moving 3-Hz frequency window (α=0.05). We found significant differences in the low alpha frequency band (7-10 Hz) at various channels across the scalp between the loaded and unloaded conditions both when participants were performing the cognitive task and walking in isolation. Specifically, alpha power was higher for the unloaded compared to loaded conditions. However, no alpha power differences were present when comparing walking while performing the cognitive task to walking in isolation for either the loaded or unloaded conditions. We believe that the decrease in alpha power resulting from carrying a heavy load is indicative of increased cortical engagement. These preliminary results suggest that walking with a heavy load requires more cortical involvement than walking with no external load, and this involvement is unaffected by a

secondary cognitive task. Further investigation using source analysis will help to identify which cortical areas contribute to these changes.

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Poster

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Topic: E.04. Voluntary Movements

Support: WSU UROP Grant 2015

Title: Degree of right-handedness mediates inter-hemispheric connectivity of the human motor cortex during inter-hemispheric transfer: fMRI evidence

Authors: *P. PATEL[1], M. BELLANI[2], K. RAMASESHAN[3], G. RAMBALDELLI[5], C. MARZI[6], P. BRAMBILLA[7], V. DIWADKAR[4];
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Abstract: Background: Approximately 90% of adults are right handed, and in these individuals, the primary motor cortex (M1) shows strong contra-lateral organization (Amunts et al., 1999). Contra-lateral organization is also a characteristic of the human visual system (Pool et al., 2014). Inter-hemispheric transfer (IT) can be probed using visuo-motor integration tasks such that visual stimulation in one hemisphere must be signaled by motor responses in the opposite hemisphere. Because the degree of handedness is related to the organization of the brain's motor system, handedness might have strong signatures in brain network connectivity during IT.
Methods: To assess IT, fMRI data were acquired using the Poffenberger paradigm (PP) in 40 healthy, right-handed subjects (3T Siemans Allegra), where handedness was established using the Edinburgh Handedness Inventory (EHI). The PP induces IT by brief tachistoscopic presentation of a visual probe to one visual hemisphere (e.g. right V1), while requiring participants to signal detection using the hand represented in the opposite hemisphere (i.e., right hand, Left M1) or the same hemisphere (i.e., left hand). Connectivity was estimated using psychophysiological interaction (PPI). PPIs were constructed by extracting time series from peak voxels. The time series was then convolved with the inter-vs-intra context of the task to indicate connectivity of the responding motor cortex. Regression analysis was conducted to determine the

relationship between degree of handedness and connectivity ($p<.05$, cluster level).
Results: For right-hand responses, a *decrease* in the degree of right-handedness predicted *increased* connectivity within LM1 (i.e. the responding M1). These effects evinced similarities, but also differences with left-hand responses. Here, decreased right-handedness predicted increased connectivity with *both* the LM1 and RM1 (i.e., responding M1).
Conclusion: These results suggest that network profiles of the motor cortices during IT (a sensorimotor context that specifically induced inter-hemispheric interactions) are strongly predicted by the degree of handedness. That decreased right-handedness (i.e., decreased lateralization) results in increased connectivity (regardless of response hand), is broadly consistent with structural MRI studies suggesting that decreased lateralization (handedness) is associated with increased thickness of callosal sub-regions (Luders et al., 2010). Assessing the generalizability of these effects (using age-matched left-handed subjects) is a focus of our continuing work.

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Poster

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Topic: E.04. Voluntary Movements

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NIH R01 NS059909

Title: Distribution of interhemispheric structural connections between motor regions on the corpus callosum in older adults

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Abstract: Many studies have shown a tendency for bilateral brain activation during movement in older adults, suggesting an increased importance of interhemispheric structural connections with age. While several methods are available to parcel the corpus callosum (CC) for

investigation of interhemispheric connections, these methods have not been validated in older adults. The purpose of this study was to determine the distribution of interhemispheric structural connections between motor regions on the CC in older adults and examine how this distribution fit within two methods of CC segmentation. Eighteen older, nondisabled adults (65 ± 9 years of age) completed a single MRI session that included diffusion tensor images (32 directions) and a T1 structural image. Previously published masks of motor cortical regions (M1, S1, PMd, PMv, SMA, preSMA) were transformed into native space and used to draw interhemispheric connections between homologous regions through the CC in FSL. Tracts were thresholded and transformed into MNI space for analysis. CC masks were drawn according to two published segmentation approaches: Hofer & Frahm (2006) and Chao et al (2009). Individual and mean tracts were compared to these callosal masks to determine whether the tract crossed the CC within the appropriate mask. Interhemispheric tracts crossed the CC in the expected pattern (from posterior to anterior: S1, M1, SMA, PMd, preSMA, PMv). Premotor connections crossed the CC in two distinct pairs: SMA/PMd, preSMA/PMv. On average, M1-M1 and S1-S1 tracts fell within the motor and sensory masks respectively, although they were at the posterior edge of the mask for one method (Hofer & Frahm) and the anterior edge for the other method (Chao). For premotor regions, the results were more variable. Using the Chao method, the average location that each tract crossed the CC fell within the premotor mask for all interhemispheric premotor tracts. Using the Hofer & Frahm method, the preSMA-preSMA and PMv-PMv tracts fell within the premotor masks but the SMA-SMA and PMd-PMd tracts did not; these tracts crossed in the motor mask and at the edge of the motor-premotor masks, respectively. Examination of individual participant data revealed a similar pattern. Interhemispheric structural connections between motor regions in this group of older adults had a similar distribution on the CC as a previous study in young adults (Fling et al, 2013). While masks drawn on the CC provide a quick assessment of the integrity of interhemispheric structural connections, further validation of these segmentation approaches is needed in older adults and clinical populations, especially for premotor connections.

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Poster

437. Cortical Planning and Execution: MRI

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 437.08/VV14

Topic: E.04. Voluntary Movements

Title: Cortical and network reorganization after bilateral forearm transplantation: a longitudinal case study

Authors: *C. R. HERNANDEZ-CASTILLO[1], J. DIEDRICHSEN[2], E. AGUILAR-CASTANEDA[3], M. IGLESIAS[4];
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Abstract: Cortical reorganization after amputation is a well-known phenomenon resulting in decreased sparse cortical activity of the respective cortical territory of motor and sensory regions. In human amputees the representation of the unaffected muscles expands to cover regions previously dedicated to the missing limb. After transplantation of a new limb, the organization of the cortical representation in the sensory and motor cortices shift again, suggesting a possible reversibility mechanism. However, there is a lack of information about the reversibility of the abnormalities at the network level after transplantation and successful rehabilitation. The objective of this study was to characterize the functional connectivity changes between the cortical territory of the new hand and two intrinsic network of interest: the sensorimotor network (SMN) and the default mode network (DMN) of one patient whom received bilateral forearm transplants. The patient was a 52-year-old man who suffered a high-voltage electrical burn causing the loss of his hands. One year after the patient underwent bilateral forearm transplantation surgery. Using resting-state fMRI we identify these two networks four months after the transplantation surgery and during three consecutive years while the patient underwent physical rehabilitation. The topology of the SMN was disrupted at the first acquisition. Through the years the SMN shifted to its canonical shape. Analysis of the DMN showed no significant changes in its topology over time. Functional connectivity between the missing hand's cortical territory and the SMN increased over time. Accordingly, the correlation of resting state time series between the missing hand's territory and the DMN decreased across acquisitions. Our results show that after transplantation a new reorganization occurs at the network level, supporting the idea that extreme behavioral changes can affect not only the local rewiring but also the intrinsic network organization in neurologically healthy subjects. Overall this study provides new insight information on the complex dynamics of the brain organization.

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Poster

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Program#/Poster#: 437.09/VV15

Topic: E.04. Voluntary Movements

Support: American Legion Brain Sciences Chair, Univeristy of Minnesota

Title: Intrinsic functional organization of the human motor cortex

Authors: *P. S. CHRISTOVA, A. P. GEORGOPOULOS;
Dept Neurosci, Univ. Minnesota, Minneapolis, MN

Abstract: Introduction: The Human Connectome Project (HCP www.humanconnectome.org) provides acquisitions of whole brain functional MRI data at high resolution and high speed. The higher resolution and short repetition time (RT) greatly enhances our ability to capture the spatial and temporal variation of brain activity and connections. This let us explore, with high anatomical accuracy and specificity, detailed interactions within a specific brain area - the motor cortex. **Method:** HCP developed protocol capable of acquiring the whole brain volume at 2x2x2 mm resolution within a repetition time of 720 ms. The resting state blood oxygenation level dependent (BOLD) time series, after proper prewhitening, were cross-correlated with varying lags. For left and right motor cortex we computed all possible pairwise crosscorrelation functions (CCF with 30 positive and negative lags) between prewhitened BOLD time series of ~2000 vertices (~2 million CCFs). For each CCF we located the highest absolute CC (|CCmax|), noted its value, sign and the lag where it occurred, and the intervertex distance. We then evaluated key spatiotemporal functions, including dependencies of the signed (|CCmax|) on intervertex distance, absolute lag on intervertex distance, and signed (|CCmax|) on absolute lag. **Results:** We found that these key functions were very robust across hemispheres and participants, indicating invariant functional interaction properties of the cortical circuitry. The frequency distribution of the signed (|CCmax|) revealed a bimodal pattern consisting of separate negative and positive CCs. We found that the percentage of negative (|CCmax|) was practically identical between the left and right motor cortex for a given participant but varied substantially among participants (range: 8.4 - 46.6%), which we term inhibitory drive. We found that the prevalence of inhibition was positively correlated with the motor dexterity and negatively correlated with the motor strength of the participant. **Conclusions:** The overall inhibitory drive is correlated with the individual's motor behavior and is the neural signature of motor capacities for a particular brain. These results are in accord with our recently advanced hypothesis that the level of motor cortical inhibitory drive is intimately related to accuracy and speed of motor performance (Mahan & Georgopoulos, Front Neural Circuits 7:92, 2013; Georgopoulos, Exp Brain Res 232:2391-2405, 2014; Georgopoulos & Carpenter, Curr Opin Neurobiol 33:34-39, 2015).

Disclosures: **P.S. Christova:** None. **A.P. Georgopoulos:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIDCD Grant R01 DC002852

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Title: A general framework for quantitatively assessing neurocomputational models with functional neuroimaging data

Authors: ***A. DALIRI**[1], J. A. TOURVILLE[2], A. NIETO-CASTANON[2], F. H. GUENTHER[2]; [1]Dept. of Speech, Language, and Hearing Sci., Boston Univ., Quincy, MA; [2]Department of Speech, Language, and Hearing Sci., Boston Univ., Boston, MA

Abstract: Advances in neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have resulted in a greatly improved understanding of the neural mechanisms underlying human sensory, motor, and cognitive capabilities, leading to increasingly sophisticated neural models of these functions. Within the domain of speech production, for example, we have developed a large-scale neurocomputational model, called the Directions into Velocities of Articulators (DIVA) model, that provides a unified mechanistic account of acoustic, kinematic, and neuroimaging data on speech production. Functional neuroimaging has been a powerful means for evaluating and refining such models. To date, however, these evaluations have been almost exclusively qualitative. Quantitative evaluations of these models with functional neuroimaging is currently hampered by the absence of a general computational framework for (i) generating predicted functional activation from the models that can be directly and quantitatively compared to empirical functional neuroimaging data, and (ii) testing between different models and selecting a model that best fits empirical functional data. Here, we present a general computational framework to overcome both of these issues. We use data from a large fMRI database of speech production studies (n=116) to test between whole-brain activity patterns generated from DIVA and those generated from alternative models. Within this framework, the brain network responsible for a task is broken into a set of computational nodes, each of which is localized to an MNI stereotactic coordinate in the brain. Associated with each node is a computational load function that links the node's activity to a computation involving quantifiable measures from the task. The instantaneous neural activity at each location in the brain (e.g., each

voxel of an fMRI image) is then calculated by summing the contributions of all model nodes at that location, with each node treated as a Gaussian activity source centered at the node's location. The parameters of the Gaussians (i.e., spread and magnitude of activation) are optimized to produce the best fit to the functional data. A trust-region reflective algorithm for minimization of non-linear least squares is employed to optimize the parameters. Model comparisons are based on the overall fit level and number of free parameters of each model using the Akaike Information Criterion (AIC). The resulting computational framework can be applied to any model of a sensory, motor, or cognitive task by simply (i) assigning an MNI coordinate to each component of the model, and (ii) defining a computational load function for that component.

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Poster

437. Cortical Planning and Execution: MRI

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Title: Functional neuroimaging of position matching at the elbow

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Abstract: Introduction: Proprioception is our sense of the position and movement of our limbs. It allows us to know where our limbs are in space so that we can carry out coordinated movements such as reaching. Previous neuroimaging studies have identified human brain areas activated during active and passive limb motion, yet we still do not have a good understanding of the areas involved during tasks that specifically require awareness of limb position. Contralateral limb position matching tasks have been used previously to study limb position sense in healthy

and neurologically impaired humans. Here, we used functional magnetic resonance imaging (fMRI) to investigate the brain areas involved in the performance of a contralateral limb position matching task. **Methods:** Five healthy right-handed control subjects were recruited to perform a mirror-matching task at the elbow during fMRI. Subjects' arms were secured to an MRI compatible device. During the task, the experimenter moved the subject's arm (+/- 10 degrees of elbow flexion) to one of four predetermined elbow angles. After a pseudorandom delay (3-5 seconds), subjects were visually cued to mirror-match the position of the passively moved arm with the opposite arm and hold. This was repeated for 20 trials per run. Two runs were performed, testing both right and left arms. T1-weighted anatomical and BOLD-fMRI sequences were collected at 3T. We analyzed these data using a general linear model in FMRIB Software Library (FSL), and modeled group level BOLD activations for both passive and active movements. **Results:** Passive elbow movements resulted in significant BOLD activations in contralateral primary somatosensory cortex and superior parietal lobule as well as primary motor, premotor, and supplementary motor areas. These results were consistent between right and left passive arms. Active matching movements with the opposite arm resulted in significant BOLD activations bilaterally in primary somatosensory, primary motor, premotor, and supplementary motor cortices. Additionally, active matching produced BOLD activations in contralateral putamen and ipsilateral cerebellum. **Conclusions:** Our results suggest that a widespread network of sensorimotor areas are involved in processing proprioceptive information at the elbow. Passive movement primarily activated contralateral sensorimotor cortical areas. However, active position matching with the opposite arm activated these same areas bilaterally as well as subcortical and cerebellar structures. These results further our understanding of proprioceptive processing in the human brain, and the areas involved in an upper-limb position-matching task.

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Poster

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Topic: E.04. Voluntary Movements

Support: Canadian Institutes of Health Research

Title: Temporal evolution of visual and motor direction selectivity in human cortex during target representation, motor planning, and reach execution

Authors: *D. C. CAPPADOCIA[1], S. MONACO[2], Y. CHEN[1], G. BLOHM[3], J. CRAWFORD[1]; [1]Ctr. for Vision Res., York Univ., North York, ON, Canada; [2]Univ. of Trento, Trento, Italy; [3]Queen's Univ., Kingston, ON, Canada

Abstract: The human brain areas involved in reach planning have been studied extensively, but the directionally selective mechanisms for target memory, movement planning, and motor execution have not been clearly differentiated. In this study, we used an event-related fMRI design that temporally separated the major stages of memory-guided reaching and saccades into three distinct phases: target representation, motor planning, and motor execution. Subjects (N=12) fixated at midline and were shown a target to the left or right of midline. After a delay of 8 seconds (the target representation phase), subjects were instructed with an auditory cue to perform a reach or an anti-reach. This was followed by a second delay of 8 seconds (the effector-specific motor planning phase). Finally, an auditory 'go signal' prompted subjects to perform the instructed movement by reaching-to-touch a touchscreen with their right hand). Using the pro-anti instruction to differentiate visual vs. motor selectivity during movement planning, we found that only the left cuneus showed contralateral visual selectivity (selectivity based on the initial target presentation), whereas a broad constellation of left occipital (primary visual cortex, lingual gyrus & superior occipital gyrus), parietal (superior parietal occipital cortex, midposterior intraparietal sulcus, anterior intraparietal sulcus, precuneus & angular gyrus), and frontal (dorsal premotor cortex & primary motor cortex) areas showed contralateral movement selectivity (selectivity based the direction of the movement). Analysis of the time courses of these movement selective brain areas through the entire memory-planning sequence revealed early visual selectivity in most areas, followed by motor selectivity in most areas, with all areas showing a stereotypical visuomotor transition. Cross-correlation of these spatial parameters through time revealed separate functional networks for visual input, motor output, and visuomotor transformation that spanned occipital, parietal, and frontal cortex. These results demonstrate that a highly distributed occipital-parietal-frontal reach network is involved in the transformation of retrospective sensory information into prospective motor plans.

Disclosures: D.C. Cappadocia: None. **S. Monaco:** None. **Y. Chen:** None. **G. Blohm:** None. **J. Crawford:** None.

Poster

437. Cortical Planning and Execution: MRI

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Support: ISF grant 961/14

Title: Mirror symmetric movement encoding in the human motor system

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Abstract: Cortical motor control exhibits clear lateralization whereby each hemisphere controls the motor output of the contralateral side of the body. Nevertheless, neural populations in ipsilateral motor areas are active during unilateral movements and even exhibit reliable directional selectivity during reaching movements to peripheral targets. It is unclear whether this directional selectivity is the same for ipsilateral and contralateral movements when examining different brain areas in the visuomotor hierarchy. To address this question, we measured fMRI responses in multiple visuomotor system areas of the human brain while subjects performed 'out and back' reaching movements to peripheral targets with either dominant or non-dominant hand. We trained a multivariate classification algorithm to identify movement direction according to voxel-by-voxel fMRI patterns in brain regions across the visuomotor hierarchy. The classifier was trained using a subset of trials and then tested by decoding the excluded trials. The direction of movement was successfully decoded with above-chance accuracy rates in all contralateral and ipsilateral motor areas when training and testing the classifier on trials within each condition (i.e. movement preformed with the same hand). Next we trained a classifier on movements performed with one hand and tested its accuracy when decoding movements performed with the other hand. fMRI response patterns in the visual cortex enabled above-chance decoding of movement direction (i.e. target location) across hands, demonstrating an effector-invariant representation. Response patterns in the primary motor cortex, premotor cortex and supplementary motor area enabled above-chance decoding of movement direction when flipping/mirroring the movement direction across the midline (i.e., movements to left targets was accurately decoded as movement to right targets). This suggests that motor neural populations encode/represent ipsilateral and contralateral hand movements in a mirror symmetric manner. Intermediate visuomotor regions in parietal cortex showed decoding accuracies consistent with a gradient from a visual, effector-invariant, representation in the parieto-occipital cortex to a motor, mirror symmetric, representation in the posterior-parietal cortex. These results suggest that neural populations in most of the motor system encode mirrored contralateral and ipsilateral movements, perhaps due to the equivalent join torques (dynamics) of these movements.

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Poster

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Topic: E.04. Voluntary Movements

Support: KAKENHI Grant 16K12956

Hamamatsu photonics K. K.

Title: The correlations of brain activations during self- and stimuli-triggered movements with personality traits.

Authors: *K. OMATA[1], S. ITO[2], H. OKADA[2], Y. OUCHI[1];
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Abstract: Personality is based on individual differences in cognition, emotion and behavioral patterns. The personality traits reflect individual features that govern particular patterns of behavior in various situations. Conventionally, five traits (Neuroticism, Openness, Extroversion, Agreeableness and Conscientiousness) are chief ingredients that construct individual personality. One of the battery for the traits is the NEO-Five Factor Inventory (NEO-FFI), with which we are able to score the patterns of individual personality. We hypothesized that brain activations in particular behaviors may be linked to the degrees of individual NEO-FFI scores. To investigate this, we conducted a finger-tapping task that required a behavioral change using the functional magnetic resonance imaging (fMRI) and explored an assessment of the personality traits. Twenty-three healthy volunteers participated in the fMRI study using a 3T MRI scans (Philips, Ingenia). An event-related design was employed with four conditions: voluntarily and forcibly initiation and inhibition of a continuous finger tapping. The participants were asked to start or stop finger-tapping following instructions. The fMRI data were analyzed by SPM8 using the points of the behavioral shifts as regressors in the general liner model. The personality traits of participants were assessed by the NEO-FFI. Multiple regression analysis was conducted to measure the correlations between the whole brain activity at behavioral changes and five personality scores. In the voluntary stop condition, Neuroticism scores were positively correlated with the brain activity in the bilateral putamen, the left hippocampus and the right precuneus, Positive correlations between Agreeableness score and brain activity in the left middle occipital gyrus and the right superior temporal gyrus were observed. In the forcibly start condition, the brain activations in the right middle frontal gyrus, the left precentral gyrus and the right cuneus were positively correlated with the scores of Conscientiousness. The results showed significant correlations between personality traits and activations in the particular brain regions when participants executed the finger-tapping tasks. This suggests that the variance of NEO-FFI scores

across subjects reflects personal differences in the brain responses that underpin the foundation of personality. However, further investigation is needed to determine which parts of brain subserve trait-related behaviors.

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Poster

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Topic: E.04. Voluntary Movements

Support: Division of Biokinesiology and Physical Therapy, USC

Title: Cortical activation associated with automatic control of pelvic floor muscles in women

Authors: *M. S. YANI[1], J. GORDON[1], S. P. ECKEL[2], D. J. KIRAGES[1], S. ASAVASOPON[3], J. J. KUTCH[1];
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Abstract: ***Background.*** The human central nervous system automatically coactivates pelvic floor muscles with trunk and lower limb muscles. Improving our understanding of the neural centers underlying this control could improve physical therapy for pelvic floor muscle pain and dysfunction.
Objective. We have recently shown in healthy men that automatic pelvic floor muscle coactivation with gluteal muscles involves specific medial wall motor cortical regions, and we also recently discovered markers of dysfunction in these motor cortical regions in both women and men with chronic pelvic pain. Here, to link these lines of evidence, we aimed to determine if automatic pelvic floor muscle coactivation also involves motor cortical activation in healthy women.
Design. A cross-sectional study.
Methods. We used functional magnetic resonance imaging (fMRI) to measure brain activity, and electromyography (EMG) to measure muscle activity, during voluntary motor tasks involving pelvic floor muscles, gluteal muscles, and finger muscles.
Results. Using fMRI we support our hypothesis that the medial wall motor cortical regions activate in women when pelvic floor muscles automatically coactivate with gluteal muscles. These motor cortical regions strongly overlap with those we previously identified are altered in women with chronic pelvic pain. Using EMG recordings, we extend our previous findings to

provide evidence of coupled neural drive between these muscle groups.
Limitations. This cross-sectional study does not address therapeutic effects on the motor cortical regions we have identified.
Conclusions. Our findings underscore the importance of the motor cortex in automatic pelvic floor muscle control in both sexes, and now allow functional brain changes in women with chronic pelvic pain to be interpreted in the context of potential pelvic motor control dysfunction at the cortical level.

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Poster

437. Cortical Planning and Execution: MRI

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Topic: E.04. Voluntary Movements

Support: SFB/TRR 135

IRTG 1901

Title: Neural correlates of multisensory action predictions investigated with a custom-made passive movement device

Authors: *B. VAN KEMENADE, E. ARIKAN, K. PODRANSKI, O. STEINSTRÄTER, B. STRAUBE, T. KIRCHER;
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Abstract: According to the forward model, we generate predictions about the sensory outcomes of our own actions. These predicted outcomes are then compared with the actual outcomes, and in case of a mismatch, a prediction error is generated. Previous research has only used unimodal action consequences. Since most actions lead to multisensory outcomes, we investigated the neural mechanisms of comparator processes in multisensory action predictions with a novel setup, allowing the manipulation of active and passive movements. Participants performed hand movements whilst holding the grip of a custom-made passive movement device. In active trials, participants moved the device actively, while in passive trials, their hand was moved by the device. In this way, we could disentangle action predictions from general expectations about upcoming stimuli. Active and passive hand movements were recorded by a camera and were visible to the participants on a screen. This visual feedback of their actions was displayed either

in real-time, or with a variable delay (0-417 ms). The task was to judge after each trial, comprising one hand movement, whether the visual feedback was delayed or not. In some trials, a tone was added and presented during the movement. Its onset was synchronised with movement onset using a motion detection algorithm applied to the camera images. In our analysis of the fMRI data, we parametrically modulated the regressors for each condition with the amount of delay. Preliminary results show a main effect of action in right cerebellum, in which activity correlated more positively with delay in active than in passive trials. Furthermore, we found an action*modality interaction in the right insula, indicating that activity correlated more positively in bimodal compared to unimodal active conditions and showed no effects in passive conditions. This results suggests that the cerebellum has a specific role in predicting both unimodal and bimodal action consequences. By contrast, the right insula may contribute to specific multisensory predictions concerning action outcomes. These data extend previous findings and suggest the existence of multisensory predictive mechanisms mediated by cerebellum and insula.

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Poster

437. Cortical Planning and Execution: MRI

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Topic: E.04. Voluntary Movements

Support: NIH R01 Grant 1R01HD079432-01A1

Title: Neural and behavioral evaluation of face and hand video stimuli

Authors: ***E. KILROY**, L. HARRISON, S. GIMBEL, S. CERMAK, L. AZIZ-ZADEH; USC, Los Angeles, CA

Abstract: To date, social and affective neuroscientific research largely relies on static images, yet people are rarely perceived in a static-state. A database that provides a spectrum of emotional and non-emotional videos is ideal for conducting a wide range of neuroscientific studies that control for processing of facial and non-facial movement. Here we present such a database: EmStim. EmStim consists of three distinct sets of video stimuli: emotional expressions (i.e., happy), non-emotional expressions (i.e., puffed-cheeks) and hand actions (i.e., cutting paper). Seven Caucasian actors (4 female; 29-39 years) were recorded making thirty-five expressions against a black background. The hands of one male and female actor were also recorded

interacting with 100 everyday objects. To enable fMRI research, all videos were edited to 3.75 seconds and controlled for low-level visual properties. Psychometeric evaluations of emotional face stimuli were collected. Additionally functional Magnetic Resonance Imaging (fMRI) study was conducted to investigate neural differences in processing the three stimulus sets during three tasks. The three stimulus sets were displayed in a pseudo-random block design consisting of 15 (5 or each type) 15-second video blocks (3 videos/block),with each block followed by 15-seconds of rest. Neurotypical child and adolescent participants observed, imitated, and mentalized to each set of videos in separate scans. Standard preprocessing and whole-brain BOLD analyses were performed. The three tasks produced significant ($p<.05$) overlapping and non-overlapping activation in the Action Observation Network (i.e., Pars opercularis). Taken together, our results suggest that EmStim is a flexible and valuable resource for social, cognitive, and affective research. Ongoing work will continue to quantify relationships among individual differences in social and motor functioning and measurements of brain function associated with the three classes of EmStim stimuli.

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Poster

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Title: Continuous decoding of movement from fMRI in a goal-directed manual tracking task

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[1]UC Santa Barbara, Santa Barbara, CA; [2]Univ. Hosp. of Cologne, Jülich, Germany

Abstract: During continuous hand movements, properties such as the position, direction, and goal of the movement are constantly changing. How does neural activity in the motor system reflect these instantaneous changes in movement? We previously developed a decoding approach to accurately reconstruct movement during a simple continuous manual tracking task from the fMRI BOLD signal across multiple sensorimotor brain regions. Here, we extend this approach to

show that multivoxel activity in these regions contains information specific to the current position and direction of the hand, independent of the visual stimulus, during a goal-directed tracking task.
Participants ($N = 20$) continuously followed one of two visual targets moving along a circular trajectory. The targets moved at different constant speeds (16 s/cycle or 12 s/cycle) and in opposite directions. In the *No Switch* condition, participants tracked one of the two targets for the entire 144 s movement block. In the *Switch* condition, participants initially followed one target while waiting for a switch cue in the opposite target. The cue signaled the participant to make a quick, straight-line movement to the opposite target, and then immediately begin to follow that target along its circular path. fMRI data were obtained on a 3T Siemans Prisma scanner with a 3x3x3 mm resolution and a 400 ms repetition time. Hand and eye positions were continuously recorded.
We used a multivoxel regression analysis to relate the time-varying conjoint activity of voxels in regions of interest to the instantaneous angular position and movement direction of each target. This regression model was then used to decode these corresponding variables on data from separate movement blocks. We found that in the *No Switch* condition, visual regions showed decoding of both targets, suggesting general representation of the stimulus position. In contrast, decoding performance in motor regions was specific to the target currently being tracked—that is, a model trained on the 16 s/cycle target positions showed accurate decoding for the 16 s/cycle movement blocks, but not for the 12 s/cycle movement blocks (and vice versa). Crucially, this specificity extended to the *Switch* condition. Models trained on one target's positions only showed accurate decoding during the time periods when that same target was currently being tracked. Together, these results show that cortical dynamics, as measured from the fMRI BOLD signal, can be used to accurately identify continuous changes in hand movement position and direction during complex visuomotor behavior.

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Poster

437. Cortical Planning and Execution: MRI

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Topic: E.04. Voluntary Movements

Support: Dana Grant

NSF GRFP

Title: Functional MRI activity patterns in the action observation network for chronic stroke patients

Authors: *P. HEYDARI[1], S.-L. LIEW[2], H. DAMASIO[2], C. WINSTEIN[2], L. AZIZ-ZADEH[2]; [1]Brain and Creativity Inst., [2]USC, Los Angeles, CA

Abstract: The action observation network(AON) is comprised of motor regions (inferior frontal gyrus, ventral premotor cortex, and posterior parietal cortex) that are active when we make an action and when we see someone else make a similar action. Previous studies in nondisabled adults describe, during imitation (simultaneous observation and execution), regions of the brain show larger BOLD signal intensity when compared to action observation or execution. Here, we ask: in patients with stroke (described below), does AON BOLD activity follow that of nondisabled adults (highest for imitation, then execution, then observation).
In this on-going study, 13 patients with chronic, first time, left MCA stroke and mild-to-moderate UE motor impairments (Fugl-Meyer UE mean=50.3/66, range 40-63) and 13 nondisabled, age-matched subjects participated. Both groups were asked to observe, execute, and imitate left (L) and right (R) hand actions in an fMRI scanner. We investigated contrasts (observation>rest, execution>rest, imitation>rest), for various regions (Inferior Frontal Gyrus pars opercularis (IFGpo), Superior Parietal Lobule (SPL), Parietal Operculum (PO), and Precentral Gyrus (PCG)).
Analysis indicated, in patients with stroke, BOLD percent signal change for the L/nonparetic hand was highest for imitation bilaterally in IFGpo, PCG, SPL, and PO, followed by execution, then observation. BOLD percent signal change for R/paretic hand was highest for execution bilaterally in the same ROIs, however, percent signal was highest during for execution, followed by observation and finally imitation for R ROIs but execution followed by imitation and finally observation for L (ipsilesional) ROIs.In nondisabled adults, percent signal change for the L hand was highest for imitation in the L PCG. In these adults, percent signal change for the R hand was highest for imitation, followed by execution, and lastly observation for all bilateral ROIs: IFGpo, PCG, SPL, and PO.
Our preliminary analyses suggest that AON regions are engaged during action imitation, execution, and observation of UE actions in individuals with motor deficits due to stroke. Patients with stroke showed a higher ipsilesional (L) BOLD percent signal change for execution, then imitation, and lastly observation involving the R (paretic) hand. Engagement of these regions may suggest recruitment of AON is highest for the less skilled hand to perform such movements (paretic in the stroke group). Alternatively, this may be due to task difficulty. Further research is required to determine such a distinction. This information may be useful in understanding poststroke rehabilitation methods using imitation.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

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Support: NDSEG (R.M.N.)

NSF GRFP (D.S., K.S)

DARPA ElectRx Advanced Study HR0011-15-2-0006

Title: Neural dust: a wireless, mm-scale device platform for interfacing with the nervous system *In vivo*

Authors: *R. NEELY[1], D. SEO[2], K. SHEN[2], U. SINGHAL[2], E. ALON[2], J. M. RABAEY[2], J. M. CARMENA[2,1,3], M. M. MAHARBIZ[2,3];
[1]Helen Wills Neurosci. Inst., [2]Electrical Engin. and Computer Sci., UC Berkeley, Berkeley, CA; [3]Bioengineering, UCB/UCSF Joint program, Berkeley, CA

Abstract: The field of therapeutic bioelectronics uses electronic devices that interface with the nervous system to treat a rapidly expanding list of diseases and disorders. Next-generation treatment strategies rely heavily on neural recording methods to trigger and shape the parameters of therapeutic stimulation by providing detailed feedback about neural dynamics in real time. Wired systems for neural recording create problems for chronic, everyday use; therefore a fully wireless implantable system is desired. However, emerging wireless approaches based on electromagnetics struggle to power and communicate with implanted devices at sizes below the millimeter scale while maintaining power levels within established safety limits. We have previously shown that both theoretically and experimentally, ultrasound is an attractive alternative for powering and communicating with sub-millimeter scale implanted devices at biologically compatible power levels. Recently, we designed and built neural dust, a wireless, batteryless, and scalable ultrasonic backscatter system. Robust, in-house fabrication flow and custom application specific integrated circuits (ASICs) enabled miniaturization of both the motes and the interrogator. We implanted neural dust motes in rats to demonstrate high-fidelity wireless recording of neural activity *in-vivo*. We are currently working towards chronic implantation of neural dust motes. These data demonstrate the potential for ultrasound-based neural interface systems to play a central role in expanding the potential of future bioelectronics-based therapies.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: R43NS083183-01A1

Title: Reducing power consumption of dbs device using platinum-iridium coating

Authors: *A. PETROSSIANS;
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Abstract: Reliable and efficient neuromodulation devices are needed for numerous clinical neuromodulation therapies. Deep brain stimulation (DBS) therapy is a technique for treatment of movement disorders. A DBS device includes a battery powered implantable pulse generator (IPG) which is implanted underneath the clavicle and sends electrical signals through one or two extension leads to one or two linear electrode arrays that are implanted deep into the brain. Continuous electrical stimulation of deep brain structures can decrease the symptoms of movement disorders such as Parkinson's disease and essential tremor. DBS therapy is expanding to a multitude of neurological disorders. Electrodes are the interfaces that transfer the electrical signals from these devices to neural tissue. An improved electrode material will have a significant impact on the capabilities of the devices and the effectiveness of treatment by enabling more efficient charge delivery and thus longer battery life. In addition, better electrode materials will allow the use of microfabricated electrodes for chronic stimulation at higher charge density and will reduce stimulus artifact and increasing signal to noise ratio, thus improving detection of important biomarkers needed for closed loop stimulation strategies. In this study, the electrochemical impedance values, measured using some commercial implantable pulse generators, showed that current was increased during voltage pulsing. As a result, lower voltage is needed to provide the same amount of desired current amplitude for the coated electrodes. The lower voltage requirements for an implant will lead to reduced power consumption. The impedance values were collected on the coated and uncoated DBS leads using voltage pulses (3V, 80μs). The impedance measurement results showed that the mean impedance value of the uncoated leads was reduced from 241ohm to 166ohm. The electrical testing results showed that the DBS leads modified with platinum-iridium require 31% less power. Thus, implant battery life can be extended by using more efficient electrodes. In this investigation, comparison of platinum-iridium coated DBS electrodes vs uncoated electrodes showed that the power consumption of the DBS device was significantly reduced, leading to longer battery life of the device.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 438.03/WW1

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5 F32 NS73422-3

Title: Soft, mechanically brain-like composite materials improve the long-term, electrical characteristics of the neural interface

Authors: ***A. SRIDHARAN**, V. VOZIYANOV, J. MUTHUSWAMY;
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Abstract: Quality of neuronal signals recorded at the neural interface rapidly deteriorates after different durations of implantation time. In addition to abiotic material degradation factors, the mechanical mismatch between the implant and brain tissue has been hypothesized to exacerbate the inflammatory response impairing the electrode-neuron interface, leading to signal loss. In our recent studies, we have shown that soft, mechanically-compliant substrates cause lower strain and strain rates at the interface. The main objective of this study is to assess whether soft, brain-like composite materials improve the long-term, electrical and functional performance of the neural interface. Tungsten or platinum/iridium microwire arrays coated with a novel soft, brain-like silicone and carbon nanotube (CNT) composite were implanted in the somatosensory cortex of rats. For tungsten electrodes, electrical impedance characteristics and noise levels were stable (n=9 electrodes) compared to uncoated controls (n=6 electrodes) over long-term implantation conditions. 75% (12 of 16) of soft-coated platinum/iridium (Pt/Ir) electrodes in a microwire array implanted for 5 months in a rat model showed similar stability in noise levels and impedance at 1 kHz. Increased noise levels in 4 of 16 soft-coated Pt/Ir electrodes correspond to increased impedances by 5 months. Analysis of spontaneous neural activity showed significantly larger peak-to-peak amplitudes of isolated units for soft-coated, tungsten electrodes (60-150 μV) compared to controls (<60μV). Similar trends in peak-to-peak amplitudes for spontaneous units are seen for soft-coated Pt/Ir electrodes (80-100 μV). Soft-coated Pt/Ir electrodes implanted over 5 months in the barrel-cortex region also showed single unit response upwards of 200 μV in amplitude to mechanical stimulation of whiskers at 1 Hz and 2 Hz. Single and multi-unit responses to whisker stimulation showed a peak response at 40 msec post-stimulus with a

duration of ~100 msec. In contrast, SNRs obtained during spontaneous or passive periods of activity showed a mixed response across the 16 channel array over implantation time with 50% of electrodes increasing in SNR (>+0.5dB) at 5 months and 30% decreasing (-0.5dB) in SNR. Future studies will focus on improving the stability of neural signals using various metrics. This study demonstrates that brain-like, soft neural interfaces can potentially improve chronic, long-term electrical impedance and neural recordings.

Disclosures: A. Sridharan: None. **V. Voziyanov:** None. **J. Muthuswamy:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 438.04/WW2

Topic: E.05. Brain-Machine Interface

Support: CNPQ/MCTI

FINEP

ISD

MEC

Title: The influence of different impedance values in neuronal recording: a correlational study in marmoset.

Authors: *B. B. GARCIA[1], J. H. SATO[1], M. F. P. ARAÚJO[1], H. S. G. PEREIRA[1,2,3];
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Abstract: Several features influence the quality of the neuronal signal recorded from chronically implanted electrodes. Besides the intrinsic factors of the neural tissue, extrinsic factors may also affect the accuracy of the recordings. Among those, impedance is of major importance. In this study, we investigated whether the pre-implant impedance of tungsten wire electrodes was correlated with the number of units recorded by each electrode. Sixty-four microeletrodes(two arrays of 32 electrodes) were manufactured using tungsten microwires covered by an inert material surface (TEFLON), with a sharp extremity (45 degrees). Microwires of two different diameters (100 and 50 μm) were used. The former was primarily used for stimulation and recording of subthalamic nucleus (STN) and the latter for neuronal recordings of several brain

areas: motor primary cortex (M1), putamen (Put), globus pallidus internus (GPi), globus pallidus externus (GPe), ventrolateral nucleus (VL) and ventroposterior lateral nucleus (VPL) of thalamus. The impedance of each microeletrode was measured before the arrays were implanted in a male marmoset (350g). The pre-implant values of impedance varied from 50 to 860 kΩ. Two weeks after the implant, local field potentials (LFPs) and single- and multi-unit activity were recorded from the arrays while the animal was freely moving inside a plexiglass box. All procedures were approved by the Institution's Ethics Committe for Animal Use (protocol 03/2015). The neuronal data was sorted offline (Offline Sorter, Plexon, USA). A total of 54 units were recorded from the two arrays. A Pearson test was used to measure the correlation between the number of recorded units in each electrode and its pre-implant impedance. A very small correlation value (0.02; p=0.867) was obtained. These results indicate that the quality of the signal in each channel (number of units) was not significantly correlated to their impedance. Therefore, impedance variations ranging from 50 to 860 kΩ do not seem to influence the number of single and multi-unit signals recorded from chronically implanted electrodes.

Disclosures: B.B. Garcia: None. **J.H. Sato:** None. **M.F.P. Araújo:** None. **H.S.G. Pereira:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 438.05/WW3

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS062019-06

Title: Functional evaluation of a superoxide dismutase mimic coating for chronically implanted neural electrodes

Authors: *X. S. ZHENG, X. T. CUI;
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Abstract: Recent advancement in brain-machine interface (BMI) has shown promise in enabling functional restoration of individuals with limb loss. Often a metal electrode array is chronically implanted into the brain region of interest, and neural signals can be recorded and decoded to control an external prosthesis. However, due to the brain’s foreign body response, microglia near the electrode site become activated and secrete pro-inflammatory cytokines, recruiting additional macrophages, and produce cytotoxic factors such as reactive oxygen species (ROS). The presence of ROS around the implant promotes neuronal death thereby degrading neural signal

quality overtime. Superoxide dismutase (SOD) can remove superoxide (a form of ROS) through dismutation by converting superoxide to molecular oxygen and hydrogen peroxide. However due to its low stability and poor bioavailability SOD has been unsuccessful in treating neurological diseases induced by oxidative stress. SOD mimics (SODm) have shown to be neuroprotective in *in vitro* and *in vivo* models of disease influenced by oxidative stress such as Alzheimer's disease and stroke. When immobilized onto the surface of neural electrodes, SODm may reduce neuronal death around the implant. Here we evaluate the acute and chronic performance of electrodes coated with immobilizable SODm (iSODm) in adult rats. 16-channel linear silicon probes with or without iSODm coating were implanted in the motor cortex of adult male rats for up to 3 months. In acute evaluations, animals were sacrificed 1 week after electrode implantation, and immunohistochemistry was performed to compare neuronal survival around the implant. In chronic evaluations, weekly impedance was measured, and neurophysiological recordings of spontaneous motor activity were acquired while the animals were freely moving. At endpoint, all animals were sacrificed and immunohistochemistry was performed. Significantly greater neuron density in every 50 μm zone between (0 μm -- 250 μm) around the coated implants were observed after 1 week of electrode implantation. Chronically, the iSODm coating showed great promise in allowing electrodes to record high quality single units throughout the 3-month period with endpoint signal to noise ratio up to 7.91, and up to 50% in single unit yield. These results indicate the iSODm coating's efficacy in promoting neuronal health around neural electrodes and have the potential to benefit chronic neural recording for BMI or basic neuroscience research.

Disclosures: X.S. Zheng: None. **X.T. Cui:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: DARPA RE-NET IAA

FDA Medical Countermeasures

FDA Office of Chief Scientist

FDA Critical Path Initiative

Title: Neural and vascular morphological changes after chronic electrode implantation

Authors: *Y.-R. GAO[1], M. YE[1], D.-H. KIM[1], A. LOZZI[1], A. BORETSKY[1], C. G. WELLE[1,2], D. X. HAMMER[1];
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Abstract: Neuroprosthetic devices that interface with the nervous system provide control signals for prosthetic or robotic assistive devices to restore movement capabilities to patients with paralysis or amputation. Although implanted electrodes that provide this interface perform well for a certain period of time, eventually the number of neurons that can be detected declines, and device performance suffers. Factors involved in this loss of function may include biological changes to neural tissue or electrode material degradation. One important but understudied component of the tissue response is tissue perfusion through the cerebral vascular system. Robust vascular perfusion is critical to normal brain function and may provide an important biomarker of device failure. Therefore, chronic tracking of neurovascular dynamics may provide insight into the long-term performance of implanted electrodes. We conducted a longitudinal study using two-photon laser scanning microscopy (TPLSM) and optical coherence tomography (OCT) imaging in the same mouse (Thy1-eGFP) at similar time points to monitor the neural and vascular morphological changes associated with electrode implantation. Animals were prepared for long-term imaging with craniotomy and window implantation under which one, two, or four-shank Michigan microelectrodes were inserted. Vascular changes monitored by OCT were assessed for both surface and intracortical vessels. Neuronal dynamics were measured with TPLSM and analyzed separately in the dendritic tuft, apical dendrites, and soma. The responses to implanted electrodes varied significantly across animals. Electrode implantation leads to vessel dilation and flow decrease during the first few days, new vessel growth in superficial layers of cortex, followed by relative stabilization of the deeper capillary network, as compared with pre-implantation baseline. Some animals experienced traumatic mechanical damage characterized by flow drop-out and dendritic bisection/retraction. The achievable TPLSM and OCT penetration depth decreased over time more rapidly at the region nearest the electrode, as compared to the control region. In summary, we were able to monitor neurovascular changes after electrode implantation up to 4 months to evaluate cortical damage on cellular and capillary spatial resolution scales. We observed key changes to both cerebral vessels and neurons that may contribute to future methods for evaluating the safety and performance of brain implantation devices.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS062019

Title: Evaluation of neural cell adhesion molecule L1 coating for improved chronic recordings

Authors: *P. CODY[1], N. SNYDER[6], J. DU[7], R. TIEN[1], J. WILLIAMS[2], S. SUWAY[3], J. ORELLANA[8], Y. INOUE[4], T. KOZAI[1], C. LEGENAUR[5], A. SCHWARTZ[5], X. CUI[1]; [1]Bioengineering, [2]Systems Neurosci. Inst., [3]Ctr. for Neurosciences, [4]Systems Neurosci., [5]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [6]Interphase Materials, Pittsburgh, PA; [7]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China; [8]Ctr. for Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Neural probes are used in brain-machine interface based prosthetics to restore movement to paralyzed individuals. Intracortical neural electrodes provide the greatest spatiotemporal resolution compared to other recording approaches to enable optimal decoding of neural activity for prosthetic control. Inflammatory brain responses including glial scarring and neural degeneration degrade and limit the longevity of recordings thereby severely hindering the clinical potential of neural probes. Our lab has demonstrated that the neural cell adhesion protein, L1, may be covalently conjugated to silicon and parylene based neural probe surfaces to reduce inflammatory glial activation, improve neuron survival and enhance neurite outgrowth throughout a chronic period in a rat model as verified with quantitative histological measures. Chronic electrophysiological recordings from L1 coated and uncoated parylene-C insulated Utah arrays were compared in male Rhesus monkeys and rats up to 72 and 12 months respectively. Each monkey was implanted in the motor cortex with both a coated and uncoated 96 channel Utah array while each rat was implanted in the primary monocular visual cortex with either a coated or uncoated 4x4 Utah array. Coated monkey arrays exhibit significantly greater number of units than uncoated. For rats, a repeatable and established visual stimulation paradigm was used to compare evoked activity between array treatments. In addition to a battery of histological analyses, laser capture micro-dissection to assay RNA expression changes in the immediate micro vicinity of the probe is developed, and combined with immunohistochemical staining, to better elucidate the mechanism of L1's benefits. Recording performance from rat implants is quantified with single-unit yield (percent of electrode sites recording single-units), single-unit signal-to-noise amplitude ratios, and multi-unit signal-to-noise firing rate ratios. Understanding the effect and mechanism by which L1 improves neural probe performance will inform approaches to better realize the full clinical potential of neural prosthetics for treating paralysis.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

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NIH K01 1K01NS066131

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Title: Dexamethasone retrodialysis attenuates microglial response to implanted probes *In vivo*

Authors: ***T. D. KOZAI**[1,2,3,6], A. S. JAQUINS-GERSTL[4], A. L. VAZQUEZ[5], A. C. MICHAEL[4], X. CUI[1];
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Abstract: Intracortical neural probes enable researchers to measure electrical and chemical signals in the brain. However, penetration injury from probe insertion into living brain tissue leads to an inflammatory tissue response. In turn, microglia are activated, which leads to encapsulation of the probe and release of pro-inflammatory cytokines. This inflammatory tissue response alters the electrical and chemical microenvironment surrounding the implanted probe, which in turn interfere with signal acquisition. Intervention strategies like neurocamouflage L1CAM coatings have shown great promise in both rodent & primate models. Dexamethasone (Dex), a potent anti-inflammatory steroid, can be used to prevent and diminish tissue disruptions caused by probe implantation. Herein, we report retrodialysis administration of dexamethasone under in vivo two-photon microscopy to observe real-time microglial reaction to the implanted probe. Probes under artificial cerebrospinal fluid (aCSF) perfusion with or without Dex were implanted into the cortex of transgenic mice that express GFP in microglia and imaged for 6 h.

Acute morphological changes in microglia were evident around the microdialysis probe. The radius of microglia activation was 177.1 μm with aCSF control compared to 93.0 μm with Dex perfusion. Dexamethasone had a profound effect on the microglia morphology and reduced the acute activation of these cells.
Our data also showed that Dex can cause large differences in microglial morphology that were not necessarily captured by traditional microglia activation metrics. While most microglia cells generally had processes that were radially projected, in some instances the microglia around Dex eluting probes had retracted most of their processes in a near amoeboid morphology and were generally wrapped around nearby BBB structures. Beyond the role of Dex as an anti-inflammatory drug, glucocorticoids have important roles regulating metabolic, arousal, attention, cardiovascular, and homeostatic functions. The Dex related changes in metabolism may contribute to the thickening of processes and increased apical processes compared to ramified microglia, although this remains to be studied. Previously we showed loss of perfusion and evidence of ischemia around the implants. When vascular injury during probe insertion is limited, Dex may be neuroprotective due to its anti-inflammatory effect. In contrast, if key vascular structures are damaged during probe insertion, delivery of oxygen and nutrients (and removal of neurotoxic waste products) may be reduced, and Dex might exacerbate local ischemia due its associated changes in cellular metabolic rate.

Disclosures: **T.D. Kozai:** None. **A.S. Jaquins-Gerstl:** None. **A.L. Vazquez:** None. **A.C. Michael:** None. **X. Cui:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: R01 NS09587501

Title: Elucidating the role of macrophage lineage in the FBR to chronically implanted microelectrode arrays

Authors: ***B. VELAGAPUDI**, M. B. CHRISTENSEN, P. A. TRESCO;
The Univ. of Utah, Salt Lake City, UT

Abstract: Neural electrodes have the ability to help patients suffering from CNS diseases and injuries by recording electrophysiological activity of populations of neurons. However, microelectrode arrays have not achieved widespread clinical implementation. This is due to

device inconsistency and unreliability, which are largely attributed to the foreign body response (FBR). Previously, our lab has shown that the FBR is a cell-mediated neuroinflammatory reaction associated with activated microglia and/or macrophages. However, it is unclear how each cell population contributes to the FBR due to the fact that yolk-sac derived microglia are indistinguishable from bone marrow derived macrophages using traditional immunohistochemical markers. To address this issue, our group utilized transgenic mice carrying CX3CR1 (GFP labeled microglia and macrophages) and HOXB8 (Td-Tomato labelled bone marrow derived macrophages). Transgenic mice (N = 18) were implanted with Michigan-style microelectrodesand sacrificed following an indwelling period of 8 weeks. Next, all animals were transcardially perfused and immunohistological analysis of brain tissue sections and explanted microelectrodes was performed. The transgenically labelled cells colocalized with IBA-1 immunoreacitvity indicating that CX3CR1-GFP and HOXB8-Td Tomato were reliable labels for macrophages and microglia. We observed both transgenically labeled cells in non-implanted hemispheres, with non-bone marrow derived cells constituting the vast majority of the labeled population. Surrounding implant sites, we observed a dramatic increase in the incidence of bone marrow derived macrophages at the implant interface that co-localized with CD68, indicating that bone marrow derived macrophages are the primary activated cell type involved in the FBR. Retrieved electrodes also showed CD68+ and HOXB8+ cells at the surface. The FBR showed a labeling pattern that was consistent with observations made in other species. The results suggest that the M1 macrophages may have a different developmental origin than M2 macrophages.

Disclosures: **B. Velagapudi:** None. **M.B. Christensen:** None. **P.A. Tresco:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: R21 - 59311490

Title: Extracellular matrix coatings minimize the FBR to high density microelectrode arrays

Authors: ***M. POLEI**, P. A. TRESCO;
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Abstract: Utah Electrode arrays (UEAs) record single unit neuronal activity inconsistently due in part to neural tissue loss associated with implantation coupled with the foreign body response (FBR), which exacerbates the initial iatrogenic injury. Moreover, we have shown that the extent

of tissue loss, blood-brain barrier leakiness and astrogliosis is inversely correlated with single unit recording performance in rats. Recently, we have demonstrated the utility of the FDA-approved neurosurgical hemostat Avitene™, a xenogeneic extracellular matrix (ECM) material, to limit neural tissue loss and improve neuronal tissue integration following the implantation of UEAs in the rodent motor cortex. Emerging evidence suggests that cell-type and tissue-specific ECM can modulate the activation state of macrophages, which we hypothesize will lead to further improvements in biocompatibility and recording. In the search for such ECM coatings, we investigated the hemostatic and immunomodulatory potential of a variety of cell-type specific ECMs using *in vitro* assays. ECM was collected from primary astrocytes, fibroblasts, mesenchymal stem cells (MSC) and glial restricted precursors (GRP), suspended in 0.25M acetic acid and dehydration adsorbed onto the walls of 1.5mL vials. Citrated rat whole blood was collected and added to tubes. Calcium was added to initiate coagulation and the tubes inverted every 15 seconds until a stable clot formed, as indicated by the ability to fully invert tube. Time to clot formation was recorded and normalized to uncoated control vials. Additionally, primary microglia and macrophages were isolated from post-natal day 1.5 rat pups and either cultured on ECM-coated or uncoated substrates under conditions of serum activation. The morphology of the microglia and macrophages were quantified as amoeboid (activated), migratory or stellate (resting) and compared statistically. All ECMs studied showed the ability to accelerate coagulation of rat blood. Morphometric studies of brain-resident microglial and macrophage activation showed that astrocyte-derived and GRP, but not Avitene™, MSC or fibroblast ECM coatings showed enhanced potential to down-regulate the activation state of microglia and macrophages. These results suggest that a cell-type and tissue specific ECM shows enhanced immunomodulatory potential *in vitro*. We hypothesize that such coatings may be a useful for reducing the FBR to UEAs and other devices chronically implanted in the CNS.

Disclosures: M. Polei: None. **P.A. Tresco:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 438.11/WW9

Topic: E.05. Brain-Machine Interface

Title: Experimental study of invasive brain-computer interface for rodents using carbon nano-tube coated micro-electrodes

Authors: *S. JAFARIZADEH[1], V. AZIMIRAD[2], P. SHAHABI[4], J. MAHMOUDI[4], T. DEHGHAN HELAN[3];

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Abstract: This paper presents a new brain-computer interface for living rat through deep brain stimulation with Carbon Nano-tube (C N T) coated micro-electrodes. Stimulation is used to reward and train the rat in order to make it move in desired directions. Surgery is performed on a rat's brain to put micro-electrodes in M F B and S1BF areas (in left and right hemispheres). We used four bipolar implanted micro-electrodes which are C N T coated by drop coating method. C N T suspension is obtained by solvents: (CH3)2CO, CH2Cl2 and (CH2)4O. Then micro-electrodes are coated and dried at 80 °C. They are tested in -v i t r o- to enhance the accuracy of experiments. Some of C N Ts are separated form electrode. But experiments showed that there is not serious problem for this phenomenon. On the other hand, in in -v i v o- experiments, the exact locations of electrodes are tested by removing the brain. Electrical stimulation of rat is done through an isolated portable signal generator circuit (as slave) which is carried by the rat. It has a RF receiver for communication with computer and a micro-controller for signal generation. Stimulation electrodes are connected to micro-controller so that the resistant is about 100 KΩ in A C S F. Slave system is connected wireless to a computer (as master) so that an operator is able to control it. A graphic user interface is designed for ease of operation. The remote control system is implemented and tested on rat. The stimulation of both hemispheres (M F B and S1BF areas) is done simultaneously and non-simultaneously. The whole system is examined in three-day period and it showed that the brain-computer interface for rat using C N T coated micro-electrodes is the effective in practice.

Disclosures: **S. Jafarizadeh:** None. **V. Azimirad:** None. **P. Shahabi:** None. **J. Mahmoudi:** None. **T. Dehghan Helan:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: DFG, grant number EXC 1086

Title: How to get PEDOT on your neural electrodes: reliable, functionalized and homogenously coated

Authors: *L. ASPLUND, C. BOEHLER, F. OBERUEBER, S. HEIZMANN, C. KLEBER, R. HASSARATI, A. SCHOPF;
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Abstract: With the introduction of PEDOT coatings to the field of neural interfaces new perspectives have opened up. PEDOT electrochemically grown onto electrodes results in substantially lowered impedance and elevated charge injection capacity. Furthermore, biofunctionalization of PEDOT is relatively easy, both in terms of its surface modification and as a drug delivery system. For this reason PEDOT, as neural electrode, has during recent years taken the step from the hands of electrochemists to more biologically oriented research facilities. A challenge in this development is to establish coating processes for the large variety of devices of interest with an outcome that can be trusted over months of implantation. Probe surveillance in situ is rarely possible wherefore reliability is of the utmost importance. We here report on the various aspects that determine the quality of electrodeposited PEDOT. The impact of the deposition process on delamination failure, dissolution, delivery functionality and overall film quality were investigated. Prevention strategies targeting the most common failure modes were established. PEDOT was grown on a variety of common probe systems, from flexible to silicon shafts and microwires, and characterized with a broad range of techniques. Their properties for recording and stimulation were analysed considering both short and long term use. The choice of deposition parameters greatly influenced the outcome even when films appeared equal in high resolution microscopy. By an optimized process, increased homogeneity of both the bulk and the surface coverage could be achieved as well as improved electrochemical stability. By introducing an additional platinum electroplating process step reliability could be accomplished for a larger variety of substrate materials. Furthermore, by adhesion promotion layers, PEDOT could be stably anchored over at least 600 million bi-phasic pulses. These qualities also substantially influenced the drug delivery functionality of the films. In summary, we show here the importance of making the right process choices for ensuring a high quality PEDOT electrode. Substrate material was found to have strong impact on the outcome but, by introducing additional process steps, even challenging substrates could be reliably coated.

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Poster

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Title: Signal quality changes and histological tissue reactions during long-term micro-electrocorticographic recordings

Authors: *C. A. GKOGKIDIS[1,4], X. WANG[1,4], M. GIERTHMUEHLEN[1], S. DOOSTKAM[2], M. SCHUETTLER[6], J. RICKERT[6], J. HABERSTROH[3], T. STIEGLITZ[4], W. BURGARD[5], T. BALL[1];
[1]Dept. of Neurosurg., [2]Dept. of Neuropathology, [3]CEMT Exptl. Surgery, Med. Center, Univ. of Freiburg, Freiburg, Germany; [4]Dept. of Microsystems Engin., [5]Dept. of Computer Sci., Fac. of Engineering, Univ. Freiburg, Freiburg, Germany; [6]Cortec GmbH, Freiburg, Germany

Abstract: Great efforts are currently being made to develop implantable devices for clinical applications. Micro-electrocorticography (µECoG) is a promising candidate technology to acquire signals from the cerebral cortical surface due to its high spatial and temporal resolution and low invasiveness. However, little is known about the long-term characteristics of chronic µECoG recordings and tissue reactions caused by the implants, albeit this topic is of fundamental importance for long-term application in humans. To address these topics, we developed an ovine model for the testing of µECoG devices. 4 animals were implanted subdurally with electrodes on either the somatosensory or auditory and motor cortex for durations between 162 and 575 days and µECoG recordings were carried out throughout this time period. Peripheral sensory and auditory stimulation were used to elicit either somatosensory- or auditory-evoked potentials (SEPs & AEPs) and their frequency-resolved SNR was calculated. Post-explantation cortical tissue samples were extracted and examined with histopathological staining methods to detect pathological tissue reactions. In the present study, we demonstrate that SEPs and AEPs are suitable to monitor long-term signal stability of chronic µECoG recordings. Analysis of changes

of signal, noise, and SNR characteristics over several months revealed systematic changes, especially a steady decrease in both signal and noise amplitudes. SEPs and AEPs were reliably measured in the first months followed by a decline in amplitude. A significant SNR decline was observed for SEPs and a similar tendency also for AEPs. Post mortem histology revealed astro- and microgliosis and meningeal thickening by a factor of approx. 2-3, in the area beneath the implanted electrode. We propose that this substantial meningeal thickening might be the main reason for signal decline, since electron microscopy and electrochemical assessment of the electrode contacts revealed no differences between pre- and post-implantation characteristics. In summary, the present work provides data to characterize long-term signal quality of µECoG recordings combined with histological analysis important on the way to clinical applications. We are currently extending our approach to simultaneous neural recordings from motor cortex and motion capture to study movement-related information as needed for neuronal motor prostheses controlled by µECoG-based implants.

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Poster

438. Neuroprosthetics: Electrodes and Tissue

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Support: NIH Grant R0101NS062019

Title: *In vivo* 2-photon imaging of neural implants: surface modification with L1CAM camouflages devices from microglial encapsulation

Authors: ***J. R. ELES**, T. D. Y. KOZAI, N. R. SNYDER, C. F. LAGENAUR, A. VAZQUEZ, X. T. CUI;
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Abstract: Implantable brain electrodes are versatile tools in neuroscience research, brain mapping, and brain-machine interfaces for paralysis and limb loss victims. Their long-term efficacy, however, is limited by microglial and astrocytic scarring and neurodegeneration around the implant that that rapidly degrades the electrical characteristics of the recorded signal. Our group has previously demonstrated in rodents and primates that coating neural probes with a

neuron-specific adhesion protein L1CAM can "camouflage" the probe as non-foreign, which reduces glial scarring and neuronal death.
The current study uses in vivo 2-photon imaging to explore the dynamics of the L1CAM coating in real time. We implanted 5 L1CAM coated and 5 uncoated silicon Michigan Arrays in the cortex of 10 mice with fluorescently-labeled microglia (GFP) for at least 6 hours. Immediately after implantation, microglia within 150 µm of either L1CAM coated or control electrode arrays extended processes toward the implants. Despite similar patterns of extension, there was significantly less process encapsulation of the L1CAM coated probe's surface (Fig1). Further, microglia within 50µm of the L1CAM coated implant were significantly less reactive.
These results highlight the effectiveness of surface modifications on neural implants to influence the early host-tissue response. This demonstrates that, while microglia extend processes as a first response to implantation trauma, their phenotype is modulated by direct contact with bioactive surfaces. These results ultimately demonstrate the acute mechanisms of L1CAM coating, which has shown promising longevity in 2.5 year primate studies.

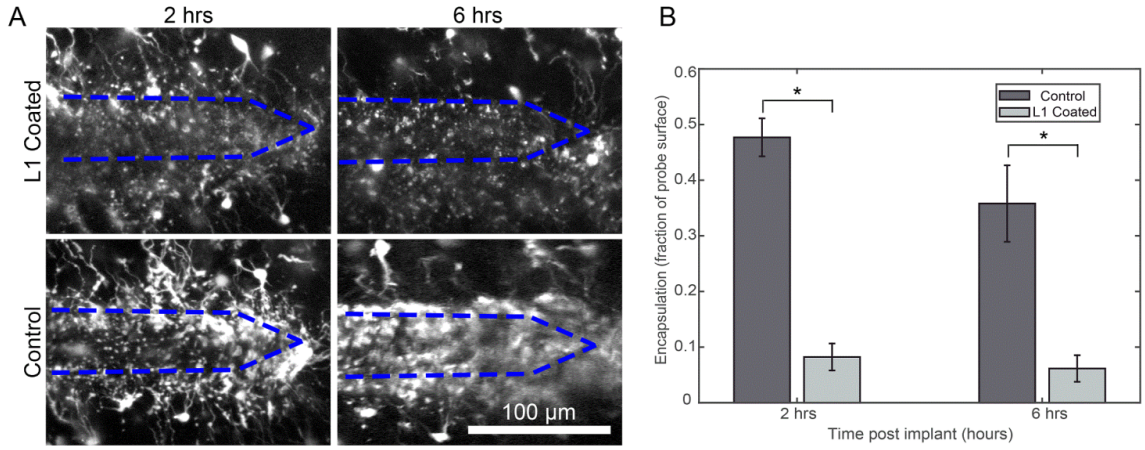

Figure 1: L1CAM coating reduces microglial encapsulation at 2 and 6hrs post implant. A) Two-photon microscopy of L1CAM coated (top) and uncoated (bottom) electrode arrays (outlined in blue) implanted in transgenic mice enable real-time imaging of microglial response to implant. B) Threshold-based quantification of microglial reveals significant reduction of encapsulation on L1CAM coated probes (Mean +- SEM; $p < 0.05$).

Disclosures: **J.R. Eles:** None. **T.D.Y. Kozai:** None. **N.R. Snyder:** None. **C.F. Lagenaur:** None. **A. Vazquez:** None. **X.T. Cui:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 438.15/WW13

Topic: E.05. Brain-Machine Interface

Title: Multicolor genetically-encoded calcium-sensitive bioluminescent reporters of neural activity for brain-machine interfaces

Authors: *M. A. PENDER[1], K. LIN[1], A. BARES[1], E. DING[1], M. G. KAPLITT[2], C. B. SCHAFFER[1], N. NISHIMURA[1];
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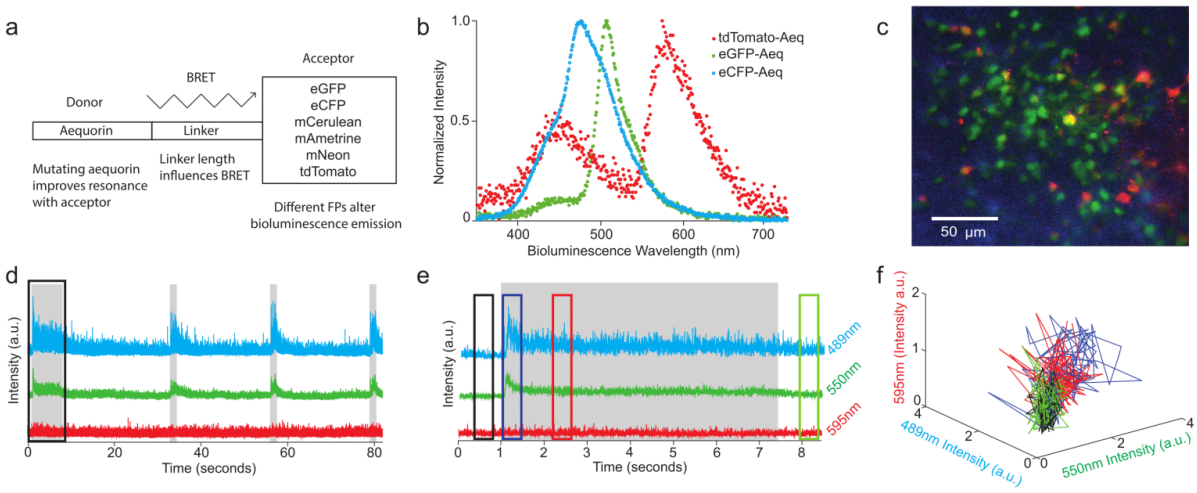
Abstract: Next-generation brain machine interfaces (BMIs) for applications such as prosthetics require the ability to map activity of neural networks over large areas, should maintain the sensitivity and resolution to detect firing of a single neuron, and must be durable. Current BMIs based on implanted electrodes can record activity from individual neurons, but cannot accurately record more than a few neurons per electrode, and degrade over time. Our approach measures neural activity by light emission from calcium-sensitive bioluminescent proteins known as fluorescent protein-aequorins (FP-Aeqs). We “barcode” neurons using a gene-therapy viral vector approach to express assortments of different color FP-Aeqs. In FP-Aeqs, aequorin acts as a calcium-sensitive donor for bioluminescence resonant energy transfer (BRET) to an acceptor connected by a flexible linker. To expand FP-Aeq’s emission spectrum, we modify and/or swap the FP, engineer mutations/truncations in the linker, and modify known interactive regions (Fig. 1a b). Selected constructs are packaged into adeno-associated viral (AAV) vectors and co-injected into rodent barrel cortex. At least three weeks after injection, multiphoton microscopy is used to directly excited FP-Aeq, confirming that a mix of AAV vectors stochastically produced different barcodes in individual neurons (Fig. 1c). Then, the cofactor required for the bioluminescent reaction, coelenterazine, is micro-injected through a craniotomy into the rodent brain. Using photomultiplier tubes, we detected spectrally separated bioluminescent emission from an anesthetized mouse in response to whisker stimulation (Fig. 1d e). Trajectories of intensity at different wavelengths showed different states before, during, and after whisker stimulation (Fig. 1f). Calcium-sensitive bioluminescence enables recording of neural activity from rodent cortex. Color-coding increases the complexity of the information relative to single-color reporters. This tool could provide a long-term, robust BMI for patients to enable control of prosthetic devices.



Disclosures: **M.A. Pender:** None. **K. Lin:** None. **A. Bares:** None. **E. Ding:** None. **M.G. Kaplitt:** None. **C.B. Schaffer:** None. **N. Nishimura:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1 R21 NS094900-01

Department of Electrical and Computer Engineering, Michigan State University

Department of Biomedical Engineering, Michigan State University

Title: Plasticity in the excitability of neurons surrounding implanted neuroprostheses

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Abstract: Implanted microelectrode arrays in the brain ("neuroprostheses") provide a unique opportunity for studying and treating neurological injury and disease by directly recording or modulating neuronal activity. However, suboptimal integration with the brain has been reported, where loss of neuronal density and glial encapsulation plague long-term devices (Biran 2005). However, evidence of a direct correspondence of these observations to functional outcomes

remains incomplete. Here, we investigate the hypothesis that alterations in the excitability of neurons surrounding implanted devices contribute to variable signal quality, where the functional properties of the affected neurons are modified as a function of distance and time from the site of insertion. Initial evidence for these effects was collected from non-functional, single shank microelectrode arrays (Neuronexus) implanted in the primary motor cortex of adult female Sprague-Dawley rats (Charles River) for predetermined timepoints (3 days, 1 week, 4 weeks), where brains were fixed with paraformaldehyde, sectioned, stained using immunohistochemistry, imaged with an Olympus Fluoview 1000 Confocal Microscope, and analyzed using a custom-modified MATLAB script adapted from (Kozai 2014). Antibodies against voltage-gated sodium and potassium channels were chosen to study fluctuations in the expression of ion channels in neurons identified by NeuN labeling. We observed initially increased ion channel expression (potentially indicating hyperexcitability) during early time points (3 days, 1 week), followed by a subsequent reduction in channel expression (a possible indicator of hypoexcitability) at the chronic time point (4 weeks) within the recordable radius of the device-interface (Henze 2000). These results were coupled with observations of shifts in the relative expression of markers of inhibitory and excitatory neurotransmission surrounding the device, where long-term time points were associated with increased inhibitory marker expression. Combined, these results support the hypothesis that local shifts in the excitability of neurons may contribute to the reported instability in recording quality over time, which may inform new strategies for improving the long-term performance necessary for clinical and research applications. Future work will correlate our findings with the quality of recordings collected from functional devices. ***References:*** **R. Biran et. al / Experimental neurology, 195(1) (2005) 115-126; D.A. Henze et. al / Journal of neurophysiology 84(1) (2000) 390-400; T. D. Kozai et. al / Biomaterials 35(34) (2014) 9255-9628.**

Disclosures: J.W. Salatino: None. **D.R. Moncrease:** None. **E.K. Purcell:** None.

Poster

438. Neuroprosthetics: Electrodes and Tissue

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Support: This work is funded by an interagency agreement with DARPA BTO under Program Manager Dr. Doug Weber and the Division of Biomedical Physics at the FDA

Title: Chronic recording and stimulation of rodent peripheral nerves using implanted microelectrode arrays

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Abstract: Reliable coupling of electrodes to the nervous system dictates chronic implant performance and the success of neuroprosthetic devices. Closed-loop neuroprosthetic systems can utilize implanted electrodes to record motor signals used for prosthetic control and deliver electrical stimulation to create sensory perception. The relative ease of access and simple circuitry in the peripheral nerves make them an attractive interface site for advanced neuroprostheses. Using a rat sciatic nerve model, this study aims to evaluate and compare the chronic recording and stimulation performance of microelectrode arrays with very low impedance electrodes to that of commercially available arrays.
Utah arrays (UA) were purchased from Blackrock Microsystems and modified Utah arrays (mUA) were provided by Dr. Loren Rieth. mUAs boast a very low impedance tip metallization based on IrOx, with median impedance < 10 kΩ and standard deviations of < 4kΩ (saline). Encapsulation for mUAs incorporates atomic layer deposited alumina underneath 6 μm Parylene-C. Arrays were implanted into the rat sciatic nerve and EMG arrays into the gastrocnemius and tibialis anterior muscles for 12 weeks, and tissue harvested for histology following study termination. Connector mounts were 3D printed to house Omnetics connectors attached to the electrodes for recording and stimulation. Two cranial screws were implanted over the somatosensory cortex to record stimulation-induced somatosensory evoked potentials (SSEP). Weekly broadband impedance measurements were recorded followed by electrophysiology to study recording performance. Two channels in each array were selected for studying stimulation stability. Stimulation evoked SSEPs and muscle activation was recorded weekly. Walking track tests were used to assess functional changes associated with long term implantation.
No differences in the recording capability of the UA and mUA, as measured by the number of channels with neural activity, were observed. Session-to-session variability in evoking SSEP was observed during stimulation of the nerve. Functional tests indicated nerve recovery around 2 weeks post-implantation. The arrays implanted into the nerve showed electrical and mechanical compatibility and did not impede recovery of function. However, chronic application of these devices may be impacted by session-to-session variability, which warrants further investigation.
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.

Disclosures: S. Vasudevan: None. **K. Patel:** None. **R. Sharma:** None. **R. Caldwell:** None. **L. Rieth:** None. **C. Welle:** None.

Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Topic: E.05. Brain-Machine Interface

Support: DARPA contract N66001-10-C-4056

Title: Native upper limb movement encoding by intracortical recordings in human sensorimotor cortex

Authors: *D. A. ROYSTON[1,2], S. T. FOLDES[6,2,3,7], J. E. DOWNEY[1,2], J. WEISS[3,1], S. N. FLESHER[1,2], E. TYLER-KABARA[3,1,4,5], M. BONINGER[3,1,5,7], R. GAUNT[3,1,2], J. L. COLLINGER[3,1,2,7];
[1]Bioengineering, [2]Ctr. for the Neural Basis of Cognition, [3]Physical Med. and Rehabil., [4]Neurolog. Surgery, [5]McGowan Inst. for Regenerative Med., Univ. of Pittsburgh, Pittsburgh, PA; [6]Barrow Neurolog. Inst. at Phoenix Children's Hosp., Phoenix, AZ; [7]VA Pittsburgh Healthcare Syst., Pittsburgh, PA

Abstract: After paralysis, attempted movement is known to activate primary motor (M1) and somatosensory (S1) cortex, although the latter is less well studied. Most previous work has been conducted with functional magnetic resonance imaging (FMRI) or magnetoencephalography (MEG). However, recent clinical brain-computer interface (BCI) trials, which have shown promise for improving function for people with paralysis, offer an opportunity to study human motor control at a single unit level. Here we investigated M1 and S1 activity using intracortical recordings while a person with tetraplegia attempted to perform movements of his arm and hand. Under an Investigational Device Exception, pre-surgical functional neuroimaging (PSFN) was used to implant two 88-channel microelectrode arrays in M1, one in shoulder, one in elbow/finger representation. Two 32-channel arrays were implanted in finger areas of S1. Intracortical data were collected 4, 8, and 12 months after implantation while a 28-year old male with a C5 motor/C6 sensory ASIA-B SCI attempted to move his right arm and hand along with videos of single-joint movements. Shoulder, elbow, and wrist movements could be overtly performed, while finger movements were covertly attempted. Modulated units were identified as those with significant changes in firing rate distribution during movement compared to rest ($p<0.05$, two-sample Kolmogorov-Smirnov test).We found that of all recorded units, over half displayed significant modulation during at least one movement (see Table 1). The medial motor array was dominated by shoulder-related units as in PSFN, while units on the other arrays were responsive to a number of different movements. This is particularly surprising on the sensory arrays, which were targeted to finger-related areas based on PSFN. We show that even after chronic tetraplegia, both M1 and S1 units respond to attempted movements, both covert and

overt. In general, units were broadly responsive to multiple movements. Further investigation is needed to examine specific movement parameters (speed, position, etc.) to determine if additional insight regarding unit behavior can be gained. Additionally, future work should examine the activation of S1 during sensory imagery as well as motor imagery.

	Motor Medial	**Motor Lateral**	**Sensory Medial**	**Sensory Lateral**
PSFN activity	Shoulder	Elbow/fingers	Fingers	Fingers
Mean # recorded units	88	87	38	41
Mean # significant during >= 1 task	70 (79%)	41 (47%)	21 (55%)	24 (59%)
% units significant during:				
All 9 tasks	28 ± 4%	44 ± 20%	40 ± 6%	40 ± 6%
Shoulder	90 ± 8%	75 ± 12%	71 ± 8%	75 ± 10%
Elbow	85 ± 5%	74 ± 9%	76 ± 10%	62 ± 16%
Wrist	72 ± 5%	76 ± 16%	69 ± 8%	77 ± 14%
Grasp	62 ± 6%	73 ± 10%	70 ± 16%	76 ± 9%
Thumb	73 ± 16%	79 ± 6%	82 ± 3%	77 ± 18%
Index	68 ± 8%	76 ± 8%	79 ± 8%	81 ± 5%
Middle	66 ± 10%	71 ± 15%	72 ± 5%	83 ± 10%
Ring	65 ± 3%	74 ± 14%	89 ± 10%	80 ± 8%
Pinky	67 ± 7%	75 ± 11%	84 ± 10%	73 ± 10%

Disclosures: **D.A. Royston:** None. **S.T. Foldes:** None. **J.E. Downey:** None. **J. Weiss:** None. **S.N. Flesher:** None. **E. Tyler-Kabara:** None. **M. Boninger:** None. **R. Gaunt:** None. **J.L. Collinger:** None.

Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Program#/Poster#: 439.02/WW17

Topic: E.05. Brain-Machine Interface

Support: DARPA N66001-10-C-4056

Title: Encoding of intended grasp force in primary motor cortex during brain-computer interface controlled robotic arm use

Authors: *J. E. DOWNEY[1], J. WEISS[2], A. B. SCHWARTZ[3], R. GAUNT[2], J. L. COLLINGER[2];
[1]Dept. of Bioengineering, [2]Physical Med. & Rehabil., [3]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Brain-computer interfaces (BCIs) for neuroprosthetic arm control are being developed as assistive devices for people with tetraplegia. Often signals from primary motor cortex (M1) are used to decode endpoint velocity, but M1 is also known to encode force. For BCI-controlled grasping, it is important to be able to modulate force in order to grasp objects of varying weights and fragility. We sought to determine if we could decode a BCI user's representation of grasp force during attempted grasping of virtual objects.

Under an Investigational Device Exemption, a 28 year-old male participant with chronic C5 motor and C6 sensory AIS B spinal cord injury was implanted with two microelectrode arrays in M1 and two microelectrode arrays in S1. Neural data was recorded while the participant watched a virtual prosthetic arm grasp a spherical object. Trials started with an auditory cue naming a graspable object (marshmallow, tomato, can of soup) chosen to span the range of possible grasp force representations. After a 2 s planning period he attempted to grasp with "the minimum force required to grasp and lift the cued object". He then attempted to hold the object with the appropriate amount of force for 2 s before being cued to release it. The virtual arm automatically grasped and released the object regardless of the participant's actions. To determine whether the intended grasp force was encoded in the recorded neural activity, each channel's firing rate was averaged over a 1 s sliding window (0.2 s step size). Each window was used to train and test a naïve-Bayes classifier using leave-one-out cross validation to create a time series of classification accuracy.

Classification accuracy of the 3 objects using firing rates in M1 peaked at 88% for the 1 s window starting 0.2 ms in to the 2 s hold time, and remained above 85% for the rest of the hold time. The accuracy did not diverge from chance level until the participant began attempting to grasp the object. When the object was released, the classification accuracy fell to 62% at the end of the trial. Classification accuracy using S1 firing rates followed a similar trend, but increased

later than M1 accuracy by approximately 0.5 s and peaked at only 67%.
This study shows that graspable objects requiring a variety of forces (the participant reported relative force ratings of 1, 2, and 4) can be well classified from M1 and, to a lesser extent, S1, in a BCI user with tetraplegia. Future work will integrate this decoded information into closed-loop BCI arm control to attempt to improve the users' ability to grasp and manipulate a variety of objects.
We thank Paul Marasco and Zachary Thumser for insightful discussion regarding study design and data interpretation.

Disclosures: **J.E. Downey:** None. **J. Weiss:** None. **A.B. Schwartz:** None. **R. Gaunt:** None. **J.L. Collinger:** None.

Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Program#/Poster#: 439.03/WW18

Topic: E.05. Brain-Machine Interface

Support: NSF

Boswell Foundation

Title: Bimanual representations in electrophysiology recordings from human posterior parietal cortex.

Authors: ***S. KELLIS**[1], Y.-L. NI[2], C. KLAES[2], T. AFLALO[2], B. LEE[3,2], K. PEJSA[1], K. SHANFIELD[4], S. HAYES-JACKSON[4], B. PHILLIPS[4], M. AISEN[4,3], C. HECK[3], C. LIU[3,2,4], R. ANDERSEN[2];
[1]Div. of Biol. and Biol. Engin., [2]Caltech, Pasadena, CA; [3]Keck Hosp. of USC, Los Angeles, CA; [4]Rancho Los Amigos Natl. Rehabil. Ctr., Downey, CA

Abstract: The parietal cortex is centrally involved in sensorimotor transformations for motor outputs such as reaches and grasps. We have shown in studies of both human subjects and non-human primates (NHP) that trajectory signals of intended limb movements can be extracted from population activity in posterior parietal cortex (PPC) and used for BMI applications in which cursors or robotic limbs are controlled by the recorded neural signals. Additional signals can also be extracted from the PPC that represent higher-level or more abstract intent. Of particular interest to brain-machine interfaces is the finding from prior animal electrophysiology recordings and human brain imaging data that representations of intended movements in both the ipsilateral

and contralateral limb are found within a single hemisphere. The bilateral nature of these signals indicates a higher-level element of activity, an idea affirmed by other similarly abstract characteristics of neuronal activity such as coding of sequences of movements and the encoding of the goals of a movement. In a recent and ongoing study of a human tetraplegic subject with PPC implants we have been able to decode both trajectory and goal signals, indicating the cognitive approach to BMIs is applicable to human subjects. Here we investigate the nature of the bimanual representation in the posterior parietal cortex using intracortical electrophysiological data recorded from a human with tetraplegia. As further evidence that the posterior parietal cortex encodes high-level bimanual activity, we have identified neurons in presumed human analogues of the anterior intraparietal area and Brodmann's area 5 whose firing rates modulate during planning and execution of motor imagery of both contralateral and ipsilateral limbs. Furthermore, bimanual representations are evident across both single-unit firing rates and population-level local fields. The involvement of the parietal cortex in bimanual movement planning confirms an important characteristic of this versatile brain area, and points toward applications in neural prosthetics whereby implants in a single hemisphere could be used to provide bilateral control of neural prostheses.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

Location: Halls B-H

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Program#/Poster#: 439.04/WW19

Topic: E.05. Brain-Machine Interface

Title: Detection of phonemes, short words and phrases from single units and 12-20 Hz frequency Beta band data during overt and covert speech recorded chronically from a speaking human.

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Abstract: Recording from a speaking human allows comparison between overt and covert speech with a view to developing a speech prosthetic for locked-in subjects (mute and paralyzed, but intelligent and awake). Four Neurotrophic Electrodes were implanted into the articulatory motor area of PRK's dominant hemisphere on June 21st 2014. Amplifiers and transmitters, powered by inductive coils, were added in October 2014. Overt and covert speech was assessed during production of 39 phonemes, 290 short words and six phrases.

The recognition of *covert* speech onset is obviously important. During *overt* speech, this is achieved by using the 12-20 Hz Beta peaks that are detected at the onset and offset of phonemes (Sarmah and Kennedy 2013) and at inflection points during the vocalizations. Extrapolating from *overt* speech results to *covert* speech, we expect that the presence of Beta peaks during *covert* speech implies speech onset, inflection points and offset. The presence of these Beta peaks indicates that the peaks can provide a means to determine what is being covertly vocalized. The data indicate that by comparing the pattern of beta peaks during overt and covert vocalizations, a 'look up' table of phrases can be developed. For example, the phrases, HELLO WORLD and WHICH PRIVATE FIRM, show differences between their beta peaks due to (1) their different lengths and (2) the inter-peak intervals of the inflection. The data indicate that this is true for both overt and covert speech. These findings demonstrate that distinct phrases, of differing lengths and inter-peak intervals, can be distinguished on the basis of their beta peaks.
Beta peaks also provide a trigger for initiating decoding of single units. There are 10 trials within each session for all phonemes and phrases for overt, covert and control periods. There is an operator triggered Event Marker available to indicate approximate Speech Onset, and in the case of *overt* speech, the Event Marker is archived along with the Recorded Speech and the Single Unit data. With *covert* speech only the Event Marker and Single Unit data are available so the Beta peak is used to decide on decoding onset. Analysis of the Single Unit activity is achieved by examining bursts that follow the Beta peak. In *overt* speech the Beta peak is found to occur at speech onset. So if a phoneme or part of a phrase is determined by *overt* speech to endure for say 30 ms, the analysis for c*overt* speech begins at the Beta peak and only 30 ms is analyzed. Thus the Beta peaks are used to determine the amount and timing of data to be analyzed. We focused on four phonemes and found that the Unipho software program (written by Chad Gambrell) indicates an 80% phoneme detection rate that occurs within 200 ms.

Disclosures: **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.. **C.R. Gambrell:** None. **N. Shih:** None.

Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Topic: E.05. Brain-Machine Interface

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Eunice Kennedy Shriver National Institute of Child Health & Human Development – NICHD (R01HD077220)

MGH-Deane Institute

The Executive Committee on Research (ECOR) of Massachusetts General Hospital

Title: Towards a multi-state click decoder in intracortical brain computer interfaces

Authors: *J. G. CIANCIBELLO[1], M. VILELA[1], T. HOSMAN[1], J. SAAB[1,2,4], D. LESENFANTS[1,2,4], D. M. BRANDMAN[3,2], B. FRANCO[5], L. R. HOCHBERG[1,2,4,5,6], J. D. SIMERAL[1,2,4,5];
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Abstract: *Background*: Intracortical brain-computer interfaces (iBCI) are being developed to enhance communication and independence for individuals with motor impairments. Assisted with the BrainGate2 BCI system, individuals with quadriplegia and locked-in syndrome have previously used their intracortical neural activity to control a computer cursor (Hochberg et al., 2006; Simeral et al., 2011). As cursor velocities increase, distinguishing a click from cursor movement must occur on a faster time scale. Here we present a new click decoding strategy aimed to enhance on-screen keyboard typing performance. We hypothesize that discrete characterization of cursor motion could improve the ability to distinguish click from ongoing movement. *Methods*: BrainGate2 participant T9 (52-year-old male with amyotrophic lateral sclerosis) had two 96-channel microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT) inserted into the dominant motor cortex. Non-causal spike rates and spike power from 20-ms bins were extracted during center-out-and-back radial-8 training and testing blocks (5 sessions). The participant was instructed to imagine moving his dominant hand to control the computer cursor and closing his hand to click. Data was used to build a Kalman filter and multi-class LDA (MCLDA) classifier with unique labels for eight cursor directions (0-44°, 45-89° 90-134°, etc.) and click. The MCLDA makes a classification decision based on a maximum likelihood estimate of the click class vs. all cursor movement classes. Point-and-click control was tested on a set of 14 center-out-and-back radial-8 task blocks and one QWERTY-typing block, during which the participant copied sentences. *Results*: T9 was able to point-and-click with a hit rate of 96.4% ± 4.3 during the center-out-and-back radial-8 test blocks. In the QWERTY block T9 copied sentences using the QWERTY on-screen keyboard at a rate of 21 correct characters per minute with 94% accuracy. *Conclusion*: The MCLDA is a new approach enhancing click decoding with increased cursor velocities by separating the high dimensional neural data with respect to cursor direction. Future sessions will compare performance from gold-standard state decoding methods (binary LDA) and MCLDA approaches. Developing a reliable click signal is essential for effective long-term BCI use that can improve the quality of life for individuals with severe motor impairments.

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Poster

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NIDCD (R01DC009899)

MGH-Deane Institute

The Executive Committee on Research (ECOR) of Massachusetts General Hospital

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Title: Reducing electrical artifacts in microelectrode brain recordings during functional electrical stimulation

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Abstract: Hundreds of thousands of people live with loss of motor function due to spinal cord injury (SCI) and could benefit from a neuroprosthetic that restores arm movements. Functional Electrical Stimulation (FES) is a method of delivering current to nerves and muscles to produce movement, and has been shown capable of restoring independence to people with spinal cord injuries, enabling activities such as eating, writing, and grooming. Intracortical brain computer

interfaces (iBCIs) have been proposed as a promising command interface for FES systems. Neural activity recorded via penetrating microelectrode arrays has been shown to be related to reaching kinematics in able-bodied primates and humans with paralysis, and iBCIs have been used to infer complex movement intentions (10+ degrees of freedom) for controlling robots. iBCIs rely on precise recordings of microvolt sized signals, but FES generates relatively larger electric fields in the paralyzed limbs. Electrical artifacts during stimulation could interfere with accurate neural recordings and limit the usefulness of iBCIs for control of FES prostheses. The goal of this work is to characterize the artifacts recorded by intracortical microelectrode arrays during functional electrical stimulation in a human, and to propose ways to mitigate those artifacts for real-time iBCI control of FES. BrainGate participant T8, who has C4 SCI, received two 96-channel intracortical microelectrode arrays placed in the motor cortex. Additionally, he had 24 intramuscular stimulating electrodes and four anodes placed in the right arm for restoring movement of the paralyzed limb. We also applied temporary surface patch electrodes to stimulate a subset of the same muscles. Stimulation artifacts were present in the neural recordings during both surface and intramuscular stimulation. However, surface stimulation artifacts were on average 175x larger than baseline neural signals while intramuscular stimulation artifacts were only 3-4x larger. Typical artifact durations were 0.5 milliseconds in length and the waveforms were highly consistent across electrodes within each array (184/192 with >90% correlation). The FES artifacts increased the iBCI computed neural feature values and reduced our ability to predict intended movements. These results demonstrate that implanted FES produces substantially smaller stimulation artifacts than surface stimulation, and that the artifacts are brief and consistent, allowing a FES-iBCI to adapt during real time neural control.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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MGH-Deane Institute

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CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Idle state detection from motor cortical activity in a person with tetraplegia using an intracortical brain-computer interface

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Abstract: *Background*: Brain-computer interfaces based on intracortical recordings (iBCI) have recently allowed two participants with tetraplegia to control a commercial Android tablet (Nuyujukian et al., SfN2015). Two outstanding issues are the inability for the participants to turn the system on/off without the assistance of a caregiver, and unintended cursor actions during idle periods. Detecting the "idle state" from neural activity could address these issues by informing the system of the user's intention (or lack of intention) to control an assistive device. The system can then automatically transition between responding to volitional neural control and idling. Here, we report the ability to distinguish motor cortical activity in task-related blocks from idle intertask periods in an individual with tetraplegia using an iBCI.
Methods: The participant in this study (T9) is a 52-year-old man in the BrainGate2 trial with amyotrophic lateral sclerosis. During research sessions, neural signals were recorded from two 96-channel microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT) placed in his dominant motor cortex. Multi-unit spike rates and power in the spike band were extracted in 20ms bins for each channel during neural control in a center-out-and-back task, and during intertask rest periods. Three sessions (A to C) were used for the following offline data analysis. Features from session A were used to train a linear discriminant analysis (LDA) classifier, then applied to an independent dataset from session B in order to evaluate the effect of two parameters on classification performance: length of the classifier's sliding window (T, ranging from 0.1 to 5sec), and number of features (F, ranging from 10 to 250). Optimal T and F were selected based on the area under session B's Receiver Operating Characteristic (ROC) curve, and then tested in session C.
Results: The optimal set of parameters (with an area under the ROC curve of 0.83) was

determined to be 3 sec of neural history combined with 100 features (35 spike rate and 65 spike power features). Linear classification could distinguish idle intertask from cursor control/task periods with a positive predictive value of 81.5% and a negative predictive value of 95.9%. *Conclusion*: This study illustrates the ability to detect idle state periods from intracortical neural recordings with high accuracy in an individual with tetraplegia. Using idle state detection online could prevent undesirable cursor movements during idle periods and allow users to turn the system on and off without the assistance of a caregiver.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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MGH-Deane Institute

The Executive Committee on Research (ECOR) of Massachusetts General Hospital

Title: Adaptive threshold for point-and-click applications using intracortical brain computer interface

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Abstract: Background: The development of intracortical Brain-Computer Interfaces (iBCI) aims to help people with locked-in syndromes to communicate and interact more completely with their environment. One of the potential applications of this technology is the full control of a computer, where the system enables the user to move the cursor and click on desired areas of the computer screen. To this end, the iBCI system needs to decode the continuous desired motion in parallel with the discrete desired clicks from the user's neural activities. The decoding of the discrete click states is often performed by a classifier, which in turn thresholds a continuous state signal to distinguish between the click and non-click classes. Here we present an algorithm that takes the state signal from a classifier and constantly updates its threshold to detect desired clicks. Methods: The proposed algorithm detects desired click by identifying when the state signal crosses its threshold. The threshold is updated from a collection of state signal points that were classified as non-click. Specifically, the algorithm recomputes the statistics of the local distribution of non-click data points in real-time, then treats clicks as outliers of this distribution. To increases responsiveness in classification, the threshold is exponentially decreased when Δ consecutive positive derivatives are detected. The parameter Δ is updated as the average time of events with consecutive positive derivatives in the non-click data points. Results: The algorithm was tested online with BrainGate2 clinical trial participant (T9) who has two 96-channel microelectrode arrays in the dominant premotor gyrus (Blackrock Microsystems). A Kalman filter was used for directional control while the log ratio of likelihoods from a multiclass linear discriminant classifier was used as state signal. After the calibration blocks, participant T9 was able to use a virtual QWERTY keyboard to copy sentences for 4 blocks, each lasting 10 minutes. These 4 blocks averaged a 17.7 correct selections per minute with overall 90% accuracy. Offline analysis of the same data using the third dimension of the Kalman filter as state signal shown similar results. Conclusion: The adaptive threshold algorithm is a versatile tool that works in conjunction with two different click decoders. Moreover, because the threshold is constantly updated, the algorithm is able to adjust to non-stationarities and short transients in the state signal and correctly detect clicks. This scenario is particularly important for long recordings where an initial fixed threshold becomes inefficient as the state signal changes its range.

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Poster

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Title: Using direction-independent, movement magnitude information from motor cortex to enhance intracortical brain-computer interface performance

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Abstract: Previous work in non-human primates has suggested that neural activity in the motor cortex is correlated with movement speed independently of direction (i.e., the equation $f = a + B \cdot V + c||V||$ describes neural activity more accurately than $f = a + B \cdot V$, where f is the firing rate of a neuron, V is a hand velocity vector, and a, B and c are model coefficients). Here, we interpreted this direction-independent activity to represent the magnitude of a feedback control vector instead of movement speed (so that V now represents a feedback control vector instead of velocity). Using this interpretation, we analyzed neural activity from motor cortex while three participants in the BrainGate2 pilot clinical trial controlled a computer cursor using a brain-computer interface. We found that direction-independent, "control vector magnitude" information was strongly encoded in threshold-crossing rates and high-frequency spectral power. Including ||V|| in the tuning model increased the average variance explained by 33%, 85%, or 202% depending on the participant. Since ||V|| is linearly independent from V, neural tuning to ||V|| is not leveraged by linear velocity decoders. We present a new decoding architecture that can explicitly decode the encoded signal ||V|| and then combine it with the magnitude information also present in V that is decoded by a standard, linear velocity decoder (Kalman

filter). The new "magnitude" decoder substantially increases the signal-to-noise ratio of decoded movement speeds offline relative to a standard Kalman filter (31%, 36%, or 152% average increase depending on the participant). Online performance tests of the magnitude decoder in one participant show that it can increase the user's ability to hold still on top of a target (the cursor stays twice as close to the target center) with no sacrifice in the time taken to reach the target.

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Poster

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Title: Closed loop intracortical brain computer interface cursor control in people using a continuously updating gaussian process decoder

Authors: *D. BRANDMAN[1,2], M. C. BURKHART[3], T. HOSMAN[4], J. SAAB[4,2,7], A. A. SARMA[4,7,2], D. J. MILSTEIN[5], C. VARGAS-IRWIN[6,2], B. FRANCO[8], J. P. DONOGHUE[6,4], M. T. HARRISON[3], L. R. HOCHBERG[7,4,8];
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Abstract: Objectives: Brain Computer Interfaces (BCIs) are being designed to allow individuals with tetraplegia to control assistive communication and environmental control devices. A core component of BCI technology is the decoder that translates neural information into command signals for such devices. We developed a nonlinear, non-parametric decoding algorithm that we call the Gaussian-process discriminative Kalman filter (GPDKF). In contrast to many other non-parametric filtering approaches, GPDKF yields a closed-form solution with interpretable parameters and provides a principled approach to dealing with signal noise and non-stationarities. We then incorporated this decoder into a closed-loop asynchronously updating system (CLAUS) that re-computes parameters online based on the most recent neural data. We validated this decoding approach during closed-loop intracortical BCI use by a person with tetraplegia.
Methods: A research participant (T9) with amyotrophic lateral sclerosis received two 96-channel multielectrode arrays that were placed in the dominant precentral gyrus as part of the BrainGate2 pilot clinical trial. The participant performed BCI-enabled computer cursor tasks with the GPDKF-CLAUS system, including center-out and random target acquisition tasks.
Results: Starting with null filter parameters, T9 developed unassisted cursor control -- allowing for target acquisition in a center-out task -- within 2.5 minutes. Standard Fitts regression parameters were comparable to previously published results. Offline analyses suggested that the GPDKF was more robust to non-stationary signal behavior than a Kalman filter.
Conclusions: The GPDKF-CLAUS decoder is a novel approach to neural decoding that removes linear assumptions of neural behavior. It provides principled methods for addressing signal non-stationarities in iBCI, and preliminary findings suggest that neural control quality is at least comparable to published controls.
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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Title: Overcoming contextual differences in motor cortical neural firing patterns when controlling multiple end effector devices using an intracortical brain-computer interface (iBCI)

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Abstract: Intracortical brain-computer interfaces (iBCI) have been used by people with tetraplegia to control computer cursors, assistive robotics, and prosthetics limbs. In this study, we examined how neural encoding of direction varies when a user alternates between controlling an on-screen cursor versus a robotic arm, and whether contextual differences in control based on the effector exist. We hypothesized that combining training data from both cursor and robotic arm tasks will allow for a neural decoding filter that would effectively control either effector. A major focus of this study was to elucidate whether neural firing patterns are contextually altered by control of a "virtual" cursor versus a physical robotic arm.
As part of the BrainGate2 pilot clinical trial, a participant (see prior abstracts) was asked to control either a cursor or robotic arm. We used a two-dimensional paradigm in which the participant had to guide either a computer cursor or robotic arm over a series of targets displayed on a computer screen, and dwell over the target for a period of 500ms. In the first set of research sessions, eight uniformly sized targets were arranged in a radial configuration. In subsequent sessions, variable sized targets were placed at randomly determined locations. Both devices used the same control scheme with matching effector speeds. We calibrated multiple filters in three

open-loop calibration scenarios in which the participant observed either, i) only cursor movement, ii) only robotic arm movement, or iii) simultaneous cursor and robotic arm movement. After obtaining the baseline performance for each filter using the modality under which it was trained, we had the participant control the effector using a filter trained with data from the opposite modality (i.e. cursor control using a filter trained on robotic arm movement). We found contextual alterations in neural firing patterns occur in relation to the end modality being controlled. However, a static neural decoding filter trained on both cursor and robotic arm blocks could provide accurate control of either effector. In the random target acquisition tasks, the participant was able to reach the target with an average time of 4.4 seconds while controlling a cursor, compared to 5.5 seconds while controlling the robotic arm. Accuracy of target acquisition was 95% in cursor control compared to 97% in robotic arm control.
Although contextual differences exist in neural firing patterns based on the modality being controlled, these disparities can be overcome using a filter trained on data that includes control of both effectors.

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Poster

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CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Retrospectively supervised point-and-click decoder calibration during practical use of an intracortical brain-computer interface

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Abstract: Brain-computer interfaces (BCIs) aim to help restore independence to people with severe motor disabilities by allowing them to control a cursor on a computer screen or other effectors with neural activity. However, physiological and/or recording-related nonstationarities in neural signals can limit long-term decoding stability. High-quality neural control can be restored using decoder calibration routines in which the user is asked to move the cursor to prescribed targets so that neural activity patterns can be mapped to movement intention. However, it would be time-consuming and impractical to require users to pause use of the BCI to perform decoder recalibration routines whenever neural control degrades. We recently demonstrated computational methods that can recalibrate a decoder using data acquired during practical point-and-click control of the BCI by retrospectively inferring that the person's movement intention at each moment had been directly toward the next self-selected target. These methods were limited to directional control of the cursor. Here, we extend this principle to allow the *click* decoder to also be recalibrated using data acquired during practical BCI use. In this study, the click decoder was a simple 2-state LDA classifier running in parallel with the kinematic decoder; however, these methods can be applied to any click decoding algorithm that turns a continuous signal (such as click log-likelihood, velocity or position in the click dimension, etc.) into a binary signal (click vs. non-click). We retrospectively labeled neural data patterns as corresponding to "click" during all time bins in which the decoded click log-likelihood had been above the click threshold that was used during real-time neural control. The periods that our kinematic decoder's retrospective target inference heuristics determined to be intended cursor movement periods were retrospectively labeled as "non-click". Once these neural activity patterns were labeled, the click decoder was calibrated using standard LDA classifier training methods. Combined with real-time bias correction and baseline rate tracking during pauses in neural control, these retrospective labeling methods enabled a BrainGate participant with ALS (T9) to type whatever he wished across 11 research sessions spanning 29 days, maintaining high-performance point-and-click control without ever needing to interrupt keyboard use for explicit calibration tasks. By eliminating the need for daily calibration tasks with prescribed targets, this approach advances the potential clinical utility of intracortical BCIs for individuals with severe motor disability.

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Poster

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The Executive Committee on Research (ECOR) of Massachusetts General Hospital

Title: Evaluating force representation in motor cortex of intracortical BCI users with chronic tetraplegia

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Abstract: Background: Intracortical brain-computer interfaces (iBCIs) have emerged as a promising assistive technology for restoring hand grasping in individuals with tetraplegia. To date, most iBCIs intended for human use have utilized only kinematic information from the motor cortex. However, during natural hand grasping, the motor cortex encodes a combination of kinematic and kinetic information. Previous studies in non-human primates have investigated the feasibility of utilizing kinetic neural information, identified during executed force production, as control signals for iBCIs. Here, we further elucidate how force-related information is represented in the motor cortex in an individual with chronic tetraplegia. Specifically, we characterize the

extent of neural modulation that occurs during observed, imagined, and attempted forces. **Methods:** Participant T8 of the BrainGate2 Clinical Trial (55-year-old male, C4-level spinal cord injury) was asked to observe, imagine, and attempt producing four discrete force levels with the dominant hand. Full broadband neural recordings were obtained from two 96-channel microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT) in the dominant precentral gyrus. We characterized the modulation of two time-varying features (spike firing rates, high frequency spike powers) during force production. These features were also used as inputs to a linear discriminant analysis (LDA) classifier, to discriminate the observed, imagined, and attempted force levels offline. **Results & Conclusions:** The number of neural features tuned to force production, as well as offline discrimination performance, was greatest during attempted force, and least pronounced when force production was observed. Additionally, tuned features exhibited various temporal profiles, with some tuned to the preparatory phase of force production, others tuned to active force production, and still others tuned to both phases. These results suggest that force-related information is retained in motor cortex in individuals with tetraplegia, and that it is feasible to incorporate cortical activity during attempted force production into iBCIs that restore hand grasping function.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

Location: Halls B-H

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Topic: E.05. Brain-Machine Interface

Support: Grant-in-Aid No.26242040 for Scientific Research (A), No.16H06260 for Young Scientists (A), and No. 26861140 for Young Scientists (B) from the Japan Society for the Promotion of Science

Title: Decoding articulation by neuronal spike frequency and power spectrum recorded from human face motor cortex.

Authors: *K. IBAYASHI[1], T. MATSUO[2], N. KUNII[3], Y. ISHISHITA[3], S. SHIMADA[3], K. KAWAI[4], N. SAITO[3];
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Abstract: Brain Machine Interface (BMI) has been actively researched and is gradually being applied in clinical scene to restore human disability. Compare to movement-assist-BMI, further improvement is still expected for verbal-communication-assist-BMI. Single unit recording is one of the most promising modality, for its high temporal resolution and high specificity in encoding neural functions. Our objective in this study was to evaluate the use of spike frequency and power spectrum obtained from human face motor cortex(FM cortex) while articulating Japanese alphabet. We recorded neuronal activities from subjects with refractory epilepsy, who underwent intracranial electrode placement for the purpose of detecting the epileptic foci. For the subjects whose FM cortex was included in potentially peri-epileptogenic zone, an electrode consisting of 6 microneedle combined with 3 macroelectrodes was used to cover the FM cortex. The protocols were reviewed and approved by the institutional ethical committee of the University of Tokyo Hospital. The task was to pronounce a single Japanese alphabet displayed on a monitor, which was pseudo-randomly presented. Neuronal activity was simultaneously recorded from macroelectrodes and microneedles (Cerebus, Black Rock). A standard expectation maximization clustering was performed to obtain single unit activities (Offline Sorter, Plexon). Local field potentials (LFPs) were band pass filtered for 6 different frequency band (Delta, Theta, Alpha, Beta, Low Gamma, High Gamma), and Hilbert transformed to obtain its power spectrum respectively. Feature vector from single unit activities was produced by optimizing the bin of spike frequency. The power spectrum was also averaged across time to produce its feature vector. We constructed multiclass classifier using SLR (Sparse Logistic Regression) or SVM (Support Vector Machine), and the performance was evaluated respectively. Binominal test revealed that the decoding accuracy of vowels were significantly over chance level ($P<0.05$), and best when feature vector consisted of spike frequency alone. Though LFP power spectrum alone was not able to meet the significance level, combination of LFP power spectrum and spike frequency reached significance level. This tendency remained as the number of decoding alphabet ranged from 2 through15. Our results suggest that optimization and further investigation for feature production from FM cortex recordings could be effective in developing verbal-communication assist BMI.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Boswell Foundation

Title: Learning mechanisms in the posterior parietal cortex: A brain-machine interface study with a tetraplegic human

Authors: *S. SAKELLARIDI[1], V. N. CHRISTOPOULOS[2], T. AFLALO[2], K. W. PEJSA[2], E. R. ROSARIO[3], D. OUELLETTE[3], N. POURATIAN[4], R. A. ANDERSEN[2];
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Abstract: One of the central questions in neuroscience is how the brain learns new tasks. Previous studies have considered two potential mechanisms: *i*) neural activity modulated by learning is independent of the structure and the normal activity of the neurons (*individual-neuron* learning) and ii) neurons use the existing network structures and the activity modulation is biased by these structures (*intrinsic-variable* learning). Here, we aim to dissociate these two hypotheses by recording neuronal activity from a tetraplegic subject (C3-C4 lesion 6 years post injury) implanted with a 96 channel microelectrode array in the anterior intraparietal (AIP) cortex. We conducted brain-machine interface (BMI) experiments, where the subject controlled a computer cursor by center-out wrist movements to two peripheral stimuli (S1, S2) by modulating the activity of one neuron (the trained neuron). The preferred direction of the trained neuron was towards the location S1. In a first block of trials (BMI-pro), the subject had to increase the activity of the neuron to move to S1, and suppress the activity to move to S2. In a second block of trials (BMI-anti), we enforced an opposite stimulus-response rule, such as the firing rate should be lower for S1 and higher for S2. This rule forces the trained neuron to flip its preferred stimulus location tuning between the BMI-pro and the BMI-anti trials. To dissociate between the two hypotheses, we recorded activity from an ensemble of "untrained" neurons. Untrained neurons are not involved in the control, but they are part of the pre-existing structure that plans the movements. If the trained neuron modulates its activity independently from the pre-existing structure, the untrained neurons will not flip their preferred stimulus (*individual-neuron* learning). However, if the subject learns a new strategy to modulate the activity of the trained neuron after introducing the opposite stimulus-response rule, the untrained neurons will also flip their preferred stimulus location (*intrinsic-variable* learning). Preliminary results are in favor of intrinsic-variable learning. We also explored whether a preferred learning mechanism varies with the task complexity i.e., is individual-neuron learning pursued when the task becomes more cognitively complex to solve? To address this question, the subject had to control the activity of two trained neurons according to the imposed stimulus-response rules. Preliminary results are also in favor of the intrinsic-variable learning. Overall, humans learn to volitionally control single-neuron activity in AIP by preferentially exploring and exploiting their natural movement repertoire.

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Poster

439. Neuroprosthetics: Human Microelectrode-Based Control

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Support: DARPA BTO SPAWAR Pacific Grant/Contract No. N66001-15-C-4017

Title: Closed-loop control of a sensorized virtual prosthetic hand by a human subject after amputation

Authors: ***D. T. KLUGER**, D. M. PAGE, S. M. WENDELKEN, T. S. DAVIS, D. T. HUTCHINSON, C. DUNCAN, D. J. WARREN, G. A. CLARK;
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Abstract: The limited availability, high cost, and lack of sensors for most commercially available high-degree-of-freedom prosthetic hands hinder research of neuromuscular interfaces for prosthetic hands. Using a virtual prosthetic hand (VPH) within a virtual reality environment (VRE) helps overcome these barriers. We have developed methods to give an amputee bi-directional, closed-loop control of a VPH in real time via implanted neuromuscular interfaces. This implementation of a sensorized VPH within a VRE supports the use of our bidirectional control algorithms for next-generation prosthetic hands.
We recorded from two 96-channel Utah Slanted Electrode Arrays (USEA; Blackrock Microsystems) implanted in residual arm nerves, and from an intramuscular 32-channel EMG assembly (Ripple, LLC) in the amputated left arm of a human subject. A motor-calibration phase captured EMG and neural recordings in response to visual movement cues of a VPH. Selected signals were weighted and combined using a Kalman filter to decode intended movements. We calibrated sensory feedback by stimulating each of the 192 USEA electrodes, and asked the subject to report the nature of evoked sensations. Electrodes that evoked proprioceptive and tactile stimuli were mapped to corresponding sensors on the VPH. The VPH wrist and digits in the MuJoCo VRE (Roboti LLC) moved in response to decoded intended movements. VPH sensor activity mediated the delivery of USEA stimulation. Motion tracking of the subject's residual limb moved the VPH through virtual space.
Motor decodes granted our subject simultaneous and independent control of up to 8 degrees of freedom, while USEA stimulation evoked 110 cutaneous and proprioceptive percepts spanning the missing hand. Our subject successfully performed literature-validated Action-Research Arm

Test (ARAT) tasks and other functionality assessment tasks modeled in MuJoCo in closed-loop. The subject identified whether a large, small, or no cylinder was placed in the VPH 26/38 times ($p < 0.001$, binomial test) without visual, audio, or other somatosensory cues.
This work demonstrates that a combined EMG/peripheral nerve interface can provide bidirectional control of a prosthetic hand. The subject's performance of virtual functionality assessment tasks further shows that a VRE can be used to assess neuromuscular-interface-mediated bidirectional closed-loop prosthetic hand control. These tests were completed in a virtual space without an expensive commercially-available physical prosthesis, which presently lack sensory output. Results encourage the development of our control and feedback algorithms for similar sensorized physical prostheses.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: Coulter Foundation

DARPA N66001-16-4006

NSF GRFP to P. V.

Title: Decoding hand level prosthetic control signals from regenerative peripheral nerve interfaces in human subjects

Authors: ***P. VU**[1], Z. T. IRWIN[1], I. C. SANDO[2], P. T. HENNING[3], M. G. URBANCHEK[2], P. S. CEDERNA[2], C. A. CHESTEK[1];
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Abstract: Peripheral nerves provide a promising source for neuroprosthetic control, given their functional selectivity and relative ease of accessibility. However, current interface methods, such as intrafascicular electrodes, are limited in a clinical setting either by low signal amplitude or interface instability. Here, we address some of these issues by extracting neuroprosthetic control signals from Regenerative Peripheral Nerve Interfaces (RPNI) implanted within a person with a transradial amputation.

RPNIs are constructed by suturing a small graft of devascularized, denervated muscle to the residual end of a severed nerve. The graft then revascularizes, regenerates and becomes reinnervated by the transected nerve. Overall, RPNIs act as stable bioamplifiers for efferent action potentials and produce recordable electromyography (EMG) signals, making them a viable source for neuroprosthetic control. Here, the subject was implanted with 3 RPNIs, one on each of the median, ulnar, and dorsal radial sensory nerve, for the treatment of symptomatic neuromas. Due to the distal location of the subject's amputation, only the median and ulnar RPNIs contained motor information.

Using percutaneous fine-wire bipolar electrodes, we were able to record a 300-400μVp-p EMG signal from the median RPNI with a signal-to-noise ratio (SNR) of 24.2 during thumb flexion and a 100-120μVp-p EMG signal from the ulnar RPNI with a SNR of 5.84 during finger abduction. In addition to the two RPNIs, we recorded EMG from residual muscles: the flexor digitorum superficialis (FDS) with a 100-120μVp-p signal and a SNR of 6.30, and the flexor pollicis longus (FPL) with ~1mVp-p signal and a SNR of 47.8. The FDS signal correlated with middle finger flexion, while the FPL signal correlated with thumb flexion.

Using these signals, we successfully predicted the subject's attempted movements in real-time. A Naïve Bayes classifier was able to classify movements as either thumb, middle, or little flexion in a 100-trial session with 100% accuracy using temporal features of the EMG waveform within 100-500Hz. These predicted movements were further used to control a Touch Bionics iLimb prosthetic hand placed in front of the subject. In addition, using the same classifier offline, we were able to classify pinch grasp, thumb opposition, thumb/little opposition, or finger adduction movements with 96.4% accuracy.

For future work, nerves can be surgically subdivided into individual fascicles to construct multiple RPNIs to increase the number of degrees of freedom. In general, we have demonstrated that RPNIs may produce a sufficient signal amplitude to control hand level prosthetics in a clinical setting.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

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Program#/Poster#: 440.01/XX11

Topic: E.06. Posture and Gait

Support: Grant-in-Aid for Scientific Research (B) (No. 26289063)

Scientific Research on Innovative Areas (No. 15H01660)

Title: Intersegmental coordination of bipedally standing rat

Authors: *Y. SATO[1], T. FUNATO[1], D. YANAGIHARA[2], Y. SATO[3], S. FUJIKI[2], S. AOI[4], K. TSUCHIYA[4];
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Abstract: Human and animal use redundant joints and muscles for maintain their posture under various environments. These joints and muscles do not move independently, but certain coordination structure among motors is considered to exist. The study of coordination is important because it potentially affects the functionality of motor control. For example, coordinated motion was reported to decrease by cerebellar ataxia (Bakker et al., 2006) and this was reported to cause inability in maintaining posture (Kung et al., 2009). Another important point of the coordination is that it can be qualitatively evaluated from the measured motion. Pinter et al. (2008) examined principal component analysis (PCA) for the measured joint motion of human standing and showed that the motion is composed of two coordination patterns. They, in addition, quantitatively showed that these patterns were correlated with the motion of center of mass (COM) and its reaction.
These characteristic of coordination expects us that quantitative evaluation of coordination will reveal the functionality of motor control and its dysfunction due to ataxia. However, how such a coordination and ataxia is connected is not still uncovered. In order to approach this question, animal with ataxia is effective. In our previous studies, we have succeeded to enable the measurement of bipedally standing motion of rat and it enables detailed investigation to the change of motion due to ataxia.
In this study, in order to investigate the coordination structure of rats, bipedally standing motion of an intact rat was measured for 300 seconds. We measured each joint positions using motion capture system and extracted intersegmental coordination of bipedally standing motion using singular value decomposition (SVD). The cumulative contribution ratio showed 2 coordinative motions were dominant, especially 1st coordination accounted for about 60 percent of all the motion. Then, these coordinations were compared with the motion of COM and trunk motion. As a result, 2 coordinations were highly correlated with COM and trunk motion, respectively. Therefore, this study shows bipedally standing motion of rat is composed of two coordinated motions; motion for COM control as principal importance and trunk motion as supplemental motion.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Topic: E.06. Posture and Gait

Support: JSPS KAKENHI Grant number 16K16420

Title: Effects of visual feedback training using center of gravity along with center of pressure for static postural balance

Authors: *H. MANI[1], K. TAKEDA[2], N. HASEGAWA[2], Y. SATO[2], S. TANAKA[2], Y. SUDA[2], H. MAEJIMA[1], T. ASAKA[1];
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Abstract: Introduction
Visual feedback training indicated by a center of feet pressure (COP) in standing has been applied commonly for postural balance. The different displacement between the COP and the center of gravity (COG), the projection of the body's center of mass (COM), is more sensitive to detect the postural stability compared to the COP alone. Our previous study showed that the COP fluctuated closely and evenly around the COM in athletes with excellent postural stability. We investigated thus the effects of visual feedback training using the COG along with the COP on static postural balance.
Methods
Twenty three healthy young adults participated in this study. The participants were divisible into COP group and COG+COP group randomly. A 3D motion analysis system and a force plate were used to calculate the COM in the sagittal plane and the COP in the horizontal plane, respectively. COG in the horizontal plane is given by the force plate signals. In the training session, the participants of COP group received the real-time COP on a screen, and were required to keep the COP within the 2 standard deviations (SD) of the COP fluctuation, which was measured on a rigid surface before the training, on a form pad placed on the force plate in the anterior-posterior (AP) direction. The participants of COG+COP group received the real-time

COG in addition to COP, and were required to keep the COG within the 2 SD as well as evenly the relative displacements between COG and COP on the form pad in the AP direction. The participants performed the trial for 40 s, and the training consisted of 12 trials. In the test sessions pre-training and post-training, the participants were required to stand on the form pad as steady as possible for 60 s. The average of COP and COM displacements, COP and COM velocities, and the distance between COP and COM displacements (the COP- COM distance) in the AP direction were calculated to assess postural stability. Then, the mean absolute value of the COP- COM distance (|COP- COM|) and the absolute value of the mean COP- COM distance (COP- COM) were calculated.

Results and discussion

After the training, the mean of the COM velocity in the COG+COP group was significantly slower than that in the COP group. In addition, the mean of the COP- COM in the COG+COP group was significantly shorter than that in the COP group. Furthermore, there was a significant correlation between the COG velocity and the COP- COM. The slower COM velocity would be caused by that the COP fluctuated around the COM more evenly in the COG+COP group compared to the COP group. We conclude that the novel feedback training using the COG along with COP is more effective to improve static postural balance than that by the COP only.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Topic: E.06. Posture and Gait

Title: Task performance during a modified Sørensen test in subjects with and without chronic low back pain

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Abstract: Time to task failure (TTF) on the Sørensen test, which requires individuals to maintain the unsupported trunk in a horizontal position, predicts first-time episodes of low back pain (LBP) as well as the development of chronic LBP. However, TTF on the Sørensen test may be influenced by anthropometric characteristics (i.e. trunk mass, trunk muscle mass, trunk length) and strength of the trunk extensor muscles. Thus, we examined TTF in both healthy

controls and subjects with chronic LBP performing 1) a standard Sørensen test, and 2) a modified test that accounted for anthropometric and trunk extensor strength differences. Fifteen subjects aged 18-45 years participated in the study (8 LBP; 7 HC). The tests were performed during separate sessions separated by at least 72 hrs. In each session, subjects were positioned prone with the iliac crest aligned with the edge of the table, the pelvis securely strapped to the table, and the ankles secured using a T-bar with an embedded single DOF load cell. The trunk rested on a platform connected to a counterbalancing weight stack through a pulley system. A 6-DOF load cell fixed between the floor and the platform was used to assess trunk forces and moments. Subjects performed four maximal voluntary contractions (MVC) of the trunk extensors, with visual feedback of the extensor moment to encourage maximal effort. For the standard Sørensen test, only the weight of the platform was accounted for with the counterweight system. For the modified test, the counterweight load was set such that the participant had to elicit 30% of maximal trunk extensor moment effort to maintain the horizontal position. During both test conditions, the subject received visual feedback on trunk position from a potentiometer mounted on the platform to maintain the target trunk position. Task failure occurred when the subject could no longer maintain the target position (± 1 degree) for more than 3 seconds. The data were analyzed with a mixed model ANOVA. TTF in the control group (139 ± 13.9 seconds) was longer than in the LBP group (93 ± 13.0 seconds; $p<0.05$). Collapsed across groups, TTF in the modified test (166 ± 16.9 seconds) was longer compared to the standard Sørensen test (66 ± 6.6 seconds; $p<0.05$). Baseline vertical forces at the trunk (441± 50.1 N for control versus 460 ± 52.9 N for LBP) and hip (491 ± 56.1 N for control versus 437 ± 51.6 N for LBP) did not differ between groups. However, baseline trunk extensor moments were significantly lower in subjects with LBP than in controls (20.5 ± 5.8 versus 40.7 ± 6.26 Nm, respectively; $p<0.05$). These data suggest that differences in performance on the Sørensen could be accounted for by an inability to generate trunk extensor moment.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Topic: E.06. Posture and Gait

Support: Grant-in-Aid for Young Scientists (B) (No. 25870164)

Title: A new biomechanical interpretation of the ankle and hip strategies in balance control during human standing

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Abstract: Depending on behavioral (kinematic) context, postural responses of standing humans have been classified into two distinct strategies; i.e., the ankle and hip strategies. During quiet, unperturbed stance on a firm surface, human body behaves as a single-link inverted pendulum rotating around the ankle joint (ankle strategy). When large perturbations are imposed or when participants stand on an unstable surface, multiple coordination patterns between the ankle and hip joints are observed (hip strategy). In contrast to such conventional formulation, several researchers have shown a co-existence of the ankle and hip strategies even during quiet standing. In addition to this, Suzuki et al. (2015) have theoretically revealed that a consistent reciprocal relationship between the ankle and hip angular accelerations (i.e., hip strategy) always emerges as a mechanical consequence of passive body dynamics, regardless of joint torque patterns of the two joints. The purpose of this study was to formulate a new biomechanical interpretation of the ankle and hip strategies in balance control during human standing. Eight healthy young participants were instructed to stand quietly on a firm surface. Three-dimensional Cartesian coordinates of reflective markers attached to the ankle, hip, and shoulder joints were measured with an optical motion capture system. As expected from the theoretical consideration mentioned above, a consistent reciprocal relationship between the ankle and hip joint angular accelerations in the amplitude ratio approximately 1:3 was observed across participants. On the other hand, the coordination patterns of the ankle and hip joint torques showed relatively large variation across participants. Our results indicate that multiple joint torque patterns, which (partially) represent active neural control of the participants, result in a single stereotypical kinematic pattern due to the human body structure (length, mass, moment of inertia, and so on). We suggest that the relative contribution of the ankle and hip joint torque; i.e., amplitude ratio of the ankle and hip joint torque fluctuations, can be an alternative, meaningful index for evaluating postural control strategies during quiet standing.

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Poster

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Topic: E.06. Posture and Gait

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Edward and Barbara Bell Endowed Chair

The Farmer Foundation

Title: Use of the Cleveland Clinic-Postural Stability Index to characterize postural instability in Parkinson's disease

Authors: *S. J. OZINGA, M. M. KOOP, S. M. LINDER, T. DEY, J. L. ALBERTS; Biomed. Engineering/ ND20, Cleveland Clin., Cleveland, OH

Abstract: Background: Postural instability is a hallmark of Parkinson's disease (PD). Objective metrics to characterize postural stability are necessary for the development of treatment algorithms to aid in the management of declines in balance. The aim of this project was to validate a mobile device platform and resultant three-dimensional balance metric that characterizes postural stability. **Methods:** An iPad/iPhone mobile Application was developed, in which biomechanical data from the embedded accelerometer and gyroscope were automatically processed to characterize movement of the center of mass in the medial-lateral, anterior-posterior, and trunk rotation directions. Twenty-seven mild to moderate individuals with PD and 27 age-matched controls completed balance tasks in which the support surface, stance, and vision were altered. A postural stability metric quantifying the amplitude (peak-to-peak; P2P) of sway acceleration in each movement direction was compared between the PD and control groups. The P2P value in each direction for each individual with PD across all trials was expressed as a normalized value of the control data in order to identify individuals with PD with severe postural instability (percentile value of postural instability greater than 95^{th} percentile of controls). Lastly, the normalized P2P data in all three directions were then combined to create a balance metric, Cleveland Clinic-Postural Stability Index (CC-PSI), to quantify postural instability across all movement directions. **Results:** Patients with PD showed significantly greater postural instability compared to the control group across all balance conditions shown by the CC-PSI metric ($p<0.01$ for all tests). Additionally, within the PD group, postural instability increased across all sway directions as sensorimotor integration became more challenging as the difficulty of the postural stability task increased. **Conclusions:** Overall, the CC-PSI, which can be rapidly derived using a mobile device, provides an unbiased and systematic metric for the quantification of postural stability in PD patients. The ease of data acquisition and processing make the CC-PSI ideally suited to better understand specific postural stability declines in PD patients and assisting in its treatment.

Disclosures: S.J. Ozinga: None. **M.M. Koop:** None. **S.M. Linder:** None. **T. Dey:** None. **J.L. Alberts:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property is in place related to the methods described in this abstract..

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.06/XX16

Topic: E.06. Posture and Gait

Title: Development of a directional intervention protocol based-evidence for individuals with chronic low back pain

Authors: *A. F. CARVALHO[1,2], A. H. NOWOTNY[2,1], M. R. OLIVEIRA[2,1], L. A. STURION[2,1], N. A. SHIRABE[1], C. E. CARVALHO[1], F. K. S. BERALDO[1], F. K. S. BERALDO[1], L. C. L. CARVALHO[1], A. W. GIL[1], R. A. DA SILVA JR[2,1];
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Abstract: Low back pain is one of the most musculoskeletal disorders in the world, with higher rate of incidence and prevalence. From some guideline evidences, the acute back pain may recover spontaneously, while subacute and chronic pain needs specific care. Actually, there is no directional intervention standardized protocol in physical therapy for better management in low back pain. The purpose of this work was to develop a directional protocol of intervention for individuals with chronic low back pain. Methods: Based on a model of evaluation and intervention, individuals between 18 and 80 years, both genders, with unknown mechanical chronic low back pain were recruited voluntarily. Participants performed four assessment steps: (1) clinical history; (2) Questionnaires: pain (VAS), disability (Roland Morris), psychosomatic (fears and beliefs: FABQ); (3) functional tests (sit and rise, walking 15 meters time, back time endurance); and (4) postural balance control (on a force platform). After baseline assessment, the participants were allocated to a specific therapy protocol related to flags colors: red (acute palliative care), orange (electrotherapy + stretching), yellow (manual therapy + manipulation) green (postural control + stabilization exercises + strengthening), and white (education). Back management was performed for 60 minutes (1-week) including into 15 minutes of education + home exercises, for a total of 12 weeks. Results: A total of 32 participants (mean age 40 ± 11 years) participated, but only 13 completed to 12 weeks. 92% of the sample started in the orange flag intervention while 8% in yellow. At the end of 6 weeks, 100% of the sample was into green protocol. A significant improvement after intervention (P <0.05) were found for: pain (-55% reduction), disability (-25% on score), fears and beliefs associated with the work (- 27% on score). A significant improvement (P < 0 .05) were also found for sit and rise functional test (14%), back endurance (44% of increase), as well as for postural control paramters (in mean 20% stability increase). In conclusion, the directional intervention protocol evidence-based in

physical therapy would be effective and used on clinical practice for chronic low back pain management.

Disclosures: **A.F. Carvalho:** None. **A.H. Nowotny:** None. **M.R. Oliveira:** None. **L.A. Sturion:** None. **N.A. Shirabe:** None. **C.E. Carvalho:** None. **F.K.S. Beraldo:** None. **F.K.S. Beraldo:** None. **L.C.L. Carvalho:** None. **A.W. Gil:** None. **R.A. da Silva Jr:** None.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.07/XX17

Topic: E.06. Posture and Gait

Support: CAPES (Processo: BEX 3084/15-9

Title: Neuromuscular pattern of trunk during one-leg balance stance in individuals with and without chronic low back pain

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Abstract: The prevalence of chronic low back pain (CLBP) is higher in adults and is often associated with poor neuromuscular postural control. The purpose of this study was to compare the neuromuscular pattern of trunk in individuals with and without CLBP during a one-leg stance. Twenty volunteers, paired by sex, being 10 with CLBP (mean age 33 yrs) and 10 without nonspecific CLBP (mean age 30 yrs) participated in the study. The participants performed three 30-second trials of a one-leg stance on a force platform, while surface electromyography (EMG) measurement were obtained bilaterally on back (multifidus at L5, iliocostalis lumburum at L3), rectus abdominis and biceps femoris (hip extensor) muscles. The EMG amplitude analysis was processed by the RMS (250ms window) and normalized by the peak of activation of muscles during the balance task. Simultaneously, the centre of pressure parameters from force platform were also computed for the balance assessment. Overall, participants with CLBP presented significantly poorer balance by centre of pressure parameters ($P < 0.05$) than participants without CLBP (Effect Size: ES $d = 1.44$). Significant poorer neuromuscular activation of trunk ($P < 0.05$) was reported for CLBP (in mean range across muscles of 49% to 89%) than those without

CLBP (in mean range of 59% to 91%), especially for the back muscles investigated (multifidus: ES d = 1.0; and iliocostalis: ES d = 1.6). Not significant differences between groups were reported for activation of rectus abdominis (without CLBP = 91% vs. with CLBP = 89%) and of biceps femoris (without CLBP = 82% vs. with CLBP = 75%).The findings indicate that CLBP affects the neuromuscular postural control of trunk, especially in back muscles for the balance during one-leg stance.

Disclosures: R.A. da Silva Jr: None. **A.F. Carvalho:** None. **A.H. Nowotny:** None. **M.R. Oliveira:** None. **L.A. Sturion:** None. **N. Shirabe:** None. **P.E. de Souza:** None. **R.S. da Silva:** None. **K.P. Fernandes:** None. **E.R. Vieira:** None.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.08/XX18

Topic: E.06. Posture and Gait

Support: Virginia Horne Henry Fund, UW Madison

Title: A novel characterization of human balance control during standing with visual sensory deprivation

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Abstract: Human standing requires complex neuromuscular coordination involving integration of sensory feedback to inform the body's control strategy. The resultant control must meet the physical constraints that define balanced standing, that is, zero average translational and rotational whole-body acceleration. To study how humans meet these criteria, we examined the force at the end-effector of the body's control, the feet, during quiet unaided standing. The force of the ground on the feet (F) is the only force on the body that can be altered to keep the body upright. The sagittal-plane F was measured during unaided standing with eyes open (EO) and eyes closed (EC) to examine how neuromuscular control of balance varied with the removal of visual information. Study participants (n=11, aged 18-53 years, 5 female) stood unaided with a plate measuring 3-dimentional force under each foot for 10 5-second duration trials in each condition presented in random order. The variability and mean speed of center of pressure (CP) displacement and the coordination between CP and F direction (θ_F) were analyzed for each leg, as well as both legs combined. Standing yielded distinct coordination patterns between the EO and EC trials. The intersection point (IP) height of F vectors that result from systematic CP

versus θ_F variation in the 3.4-7.7Hz range was significantly higher with eyes closed (p=0.001 for each leg, p=0.0008 for combined legs). With eyes closed, the average combined IP increased 7.5% of body height above the average IP height of 27% body height with eyes open. CP variability and mean speed were not significantly different between the EO and EC standing conditions (p=0.5 & p=0.41, respectively). These results suggest that humans modify their neuromuscular balance strategy when faced with visual sensory deprivation. The raised IP seen with visual impairment reflects a change in coordination that would increase whole-body angular stiffness at an energetic cost. It is interesting that the CP variability and mean speed, conventional measures of standing behavior, were not different between the two conditions. The IP may characterize the ability to adapt to balance challenges such as visual deprivation, and may be useful for better understanding the disordered mechanism in those with impaired balance.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

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Program#/Poster#: 440.09/XX19

Topic: E.06. Posture and Gait

Support: University of Ottawa 119656

Title: 12 weeks of balance and mobility training with or without simultaneous cognitive training improves reaction time but does not improve posture in healthy older adults

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Abstract: Falls are a major issue for older adults, and may be caused by an impaired interaction of postural and cognitive functioning. An extensive amount of research has been devoted to improving dynamic balance via balance training; however mixed findings exist as to whether balance training improves postural control. The purpose of study was to determine whether balance and mobility training (BMT) or balance and mobility plus cognitive training (BMT+C) would reduce postural sway and reaction time and maintain these improvements after a 12-week follow-up in healthy older adults. Participants were allocated the BMT (n=15; age: 70.2 ± 3.2), BMT+C (n=14; age: 68.7 ± 5.5), or the control group (n=13; age: 66.7 ± 4.2). The BMT involved training one-on-one, 3 times per week for 12 weeks on a balance obstacle course while completing manual tasks. The BMT+C involved training one-on-one, 3 times per week for 12

weeks on a balance obstacle course while completing manual and cognitive tasks. During the testing sessions, participants stood on a force plate for 30 s in feet-apart and semi-tandem positions while completing simple reaction time and choice reaction time tasks at baseline, at the 12-week post-training, and at the 12-week follow-up. They were instructed to stand as still as possible while verbally responding as fast as possible to the stimuli. No group differences in center of pressure (COP) Area, COP Velocity, or Sample Entropy of the COP displacement in the medial-lateral or anterior-posterior directions were shown after the training or 12-week follow-up. The BMT and BMT+C showed faster reaction times after training and maintained these improvements at the 12-week follow-up compared to the control group. No differences in postural sway or RT emerged between the BMT and BMT+C groups. Both training groups improved reaction time after the interventions and sustained these improvements over time, but showed no reduction in postural. These results suggest that multi-task balance and mobility training improves attention demand in healthy older adults.

Disclosures: D.A. Jehu: None. **N. Paquet:** None. **Y. Lajoie:** None.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.10/XX20

Topic: E.06. Posture and Gait

Title: Different leaning effects of dynamic postural control by visual or auditory feedback training

Authors: *N. HASEGAWA[1,3], M. SAKUMA[4], S. TANAKA[1], Y. SATO[1], K. TAKEDA[1], H. MANI[2], H. MAEJIMA[2], T. ASAKA[2];
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Abstract: Introduction
Feedback training using sensory information such as visual, auditory or somatic senses is commonly applied to improve postural balance. Most of the previous studies concerning the feedback postural training have reported the effects of static postural control by visual feedback training. However, the differences of learning effects between visual feedback training and the other sensory feedback training are unclear. Therefore, the purpose of this study was to investigate the different learning effects by auditory feedback training compared to visual feedback training for dynamic postural control.

Methods

Eighteen healthy young adults participated in this study. The subjects were randomly separated into a visual (n = 9) or auditory group (n = 9). In test sessions, the subject was required to stand on a force plate in front of a screen, and bring his/her center of pressure (COP) by voluntary body's sway in line with the hidden target, which moved a sine-wave pattern, in the anterior-posterior (AP) direction. The target became visible on the screen in connection with a beep sound only when the target reached to the inflection points of the sine-wave. In a training session, the subject was asked to bring the COP in line with the hidden target using a visual circle on the screen, which changed in the diameter of circle according to the distance between the COP and the target, for 30 s in the visual group. In the auditory group, the subject was asked to bring the COP in line with the hidden target using a sound, which changed in the tone according to the distance between the COP and the target, for 30 s. The subject performed 40 trials on the same day. The test sessions consisted of 4 times when the time before the training (pre-test), after 20 trials (mid-test), after all trials (post-test), and 48 hours after the training (retention). The average and the standard deviation of the distances from the COP to the target (D_{ave} and D_{sd}) were calculated each trial. Two-way mixed-design ANOVA was used with factors *Group* (Visual and Auditory) and *Test session* (Pre, Mid, Post and Retention) in the indices.

Results and discussion

D_{ave} and D_{sd} showed significant reduction during the training session in each group. However, D_{sd} of the auditory group showed significant reduction compared to that of the visual group in the retention test although D_{sd} in the post test showed no significant difference between the groups. These results supported the previous study that demonstrated the different effects of motor learning of hands between visual and auditory feedback trainings. We conclude that auditory feedback training is superior to visual feedback training to learn dynamic postural control.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.11/XX21

Topic: E.06. Posture and Gait

Title: The effect of mental fatigue on postural stability

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Abstract: Proper allocation of attentional resources is required for maintaining postural stability. Research indicates that fewer attentional resources are available for balance control when individuals engage in a dual task paradigm involving concurrent performance of a cognitive task. However, these effects have not been studied under conditions of mental fatigue. To determine if mental fatigue has an effect on postural stability, center of pressure (COP) displacement in response to standing platform perturbations was recorded at the beginning and end of 20 minutes of the psychomotor vigilance task (PVT). The PVT is a sustained attention task that induces mental fatigue, as indicated by increases in reaction time to visual stimuli. Six young, healthy individuals (23.5 + 3.7 years; 5 F) participated in the study. Reaction time during PVT was significantly longer in the last 5 minutes of the task compared to the first five minutes ($p= 0.03$; $d= 0.73$), indicating mental fatigue. Center of pressure displacement in the anterior-posterior direction was significantly larger at the end of the mental fatigue task compared to the beginning of the task ($p= 0.03$; $d= 0.76$). The increase in center of pressure displacement in response to an unexpected postural perturbation suggests that mental fatigue has the functional consequence of compromising postural stability.

Disclosures: **A. Morris:** None. **A. Christie:** None.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.12/XX22

Topic: E.06. Posture and Gait

Support: JSPS KAKENHI Grant Number 15K01474

Title: Standing postural control while stepping over randomly moving virtual obstacles

Authors: *H. IDA[1], S. MOHAPATRA[2], A. S. ARUIN[3];
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Abstract: The purpose of this study was to investigate early (EPAs), anticipatory (APAs), and compensatory (CPAs) postural adjustments during control of vertical posture while stepping over a virtual obstacle. Ten healthy adults were asked to stand upright and avoid contact with an approaching virtual obstacle by lifting their left leg while maintaining their balance. This

approaching obstacle was projected virtually by a head mount display. Experiments involved virtual tasks in two different conditions: (1) steady obstacle heights (10, 20 and 30 cm) and (2) unsteady up-and-down (hopping-like) obstacle heights (up to 10, 20, and 30 cm). Surface electromyography (EMG) signals were recorded bilaterally from the tibialis anterior (TA), medial gastrocnemius (MG), rectus femoris (RF), biceps femoris (BF), rectus abdominis (RA), erector spinae (ES), and external oblique (EO) muscles. The EMG signals from the supporting (right) side were then integrated from -550 to -150 ms (EPAs), -150 to +50 ms (APAs), and +50 to +250 ms (CPAs) in relation to the moment of the initiation of the left ankle elevation (T0). Similar time windows were used for the analysis of the integrated EMG signals from the muscles on the lifting (left) side.
Two-way ANOVAs (obstacle movement × obstacle height) for the EPAs showed a significant main effect of obstacle movement for the ES muscle ($p = 0.019$) on the supporting side and MG muscle ($p = 0.047$) on the lifting side. For the APAs, a significant main effect of obstacle movement was found in the EMG signal of RA muscle ($p = 0.040$) on the supporting side. Subsequently for the CPAs, there were significant interactions between obstacle movement and obstacle height in BF muscle ($p = 0.044$) on the supporting side and in the muscles of MG ($p = 0.011$), BF ($p = 0.004$), and EO ($p = 0.003$) on the lifting side. The results indicate that the various characteristics of obstacle movement (steady or unsteady) could influence the preparatory phase (EPAs and APAs) of postural control. This phenomenon later becomes more evident during the reactive phase (CPAs) of postural control. This outcome suggests that the CNS uses early, anticipatory, and compensatory postural adjustments to maintain control of posture while stepping over virtual obstacles.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

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Program#/Poster#: 440.13/YY1

Topic: E.06. Posture and Gait

Support: JSPS KAKENHI Grant Number 25304

Title: Intermittent muscle activities occur according to joint state during human quiet standing

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Abstract: INTRODUCTION: The neural-muscular-skeletal causality of maintaining upright posture is not yet fully understood experimentally because postural control tasks are aperiodic and non-stationary. Intermittent processes of the postural control mechanism play an important role in stabilization dynamics in the vicinity of the equilibrium. Thus, we focus on the intermittent system of the postural feedback loop to visualize the causality among neural intermittent motor command triggering, muscle activities, and skeletal fluctuations. The aim of this study was to investigate the direct relationship between joint fluctuation, muscle activity and torque output.
METHODS: Eight healthy males were instructed to stand quietly on a force platform for 120 sec. Joint motions data was obtained using a 3D optical motion capture system. We also recorded surface Electromyography (EMG) over six leg muscles. We computed joint angles and torques of the ankle, knee, and hip in the sagittal plane. We also determined phasic on/off (activation/deactivation) switching of muscle activity from EMG data by using two low-pass filters, each of which represents phasic and tonic components of muscle activations.
We then divided the time history of a state point in the phase planes of each joint into on and off periods for each muscle to visualize the phasic kinetic-kinematic relationship. Each on/off area was fit into a Gaussian distribution for every muscle's on/off-period, and we plotted their centers for all trials. We did the same procedure in the torque planes.
RESULTS: Most muscles activated when the state point was located in the anatomically opposite area in the phase plane and deactivated when it was in the anatomical action direction. In addition, phasic muscle activation and deactivation were associated with torque generations in the anatomical action direction.
DISCUSSION: We experimentally visualized the causality among joint oscillations, phasic muscle activities, and torque output during quiet standing. Extracting phasic components of muscle activities allowed us to observe the direct causality, which has long been difficult to demonstrate because joint fluctuations are aperiodic and muscle activities are small and contain many frequency components during quiet standing without any disturbances. Our results will comprise fundamental knowledge of postural control closed-loop mechanisms and will be useful for evaluating balancing skills in future analysis. In conclusion, our results suggest that phasic muscle activities occur depending on the joint state in the phase space, leading to joint actuation via torque generation along with anatomical action direction.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Topic: E.06. Posture and Gait

Support: Marie Curie Integration Grant FP7-PEOPLE-2012-CIG-334201 (REMAKE)

Title: Learning postural control with continuous visual feedback in healthy young adults and chronic stroke survivors

Authors: *L. PELLEGRINO[1], P. GIANNONI[1], L. MARINELLI[2], M. CASADIO[1];
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Abstract: Visual feedback is largely used in rehabilitation to improve the control of standing or sitting posture and to train shifting weight by moving the entire body or just the trunk. Postural control deficits are common in stroke survivors and often the rehabilitation programs include balance training based on the continuous feedback of a cursor on a computer screen controlled by the position of the Center of Pressure (CoP). The goal of this study is to test if subjects are able to transfer the control of their CoP from a training protocol based on continuous visual feedback to performance without visual feedback and to directions and displacement amplitudes different from those experienced during training. We addressed the following questions: (i) if and to what extent it is possible to learn the proposed task, independently from age and/or disease, (ii) if and to what extent a population of chronic stroke survivors can learn the proposed task. To answer the first question we enrolled eleven healthy young adults (7 Female; 22±1.12 years), and to answer the second question eleven chronic stroke survivors (6 Female; 59±12 years). The subjects seated on a stool positioned on top of a custom-built force platform. The stool had a support for the feet and it was positioned on top of a force platform. The estimated CoP positions were mapped to the coordinates of a cursor on a computer monitor, placed 2 meters away from the platform at eye level. When the subject's trunk was in the upright posture, the cursor was at the center of the screen. Subjects had to reach targets by shifting their CoP. During training, the targets were presented in three different directions (0, 135 and 270 degree) at 8 cm from the center and continuous visual feedback of the cursor was provided. Then, we tested if subjects in absence of visual feedback were able to reach the same targets as well as targets in different directions (45, 90, 180, 225 and 315 degree) and with two different displacement amplitudes in the trained directions (5 and 11 cm, respectively). Healthy subjects and stroke survivors -as expected - had different performance levels, however with training both groups improved duration, smoothness and accuracy of cursor movement trajectories. In absence of visual feedback, the performance of both groups was worse with respect to the level reached at the end of training. However, while healthy subjects partially transferred the learned abilities to the no visual feedback condition most stroke survivor did not. This result suggests that a postural training based exclusively on continuous visual feedback could provide limited benefits for stroke survivors.

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Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Program#/Poster#: 440.15/YY3

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant

Title: Increased postural threat influences the conscious perception of voluntary leaning

Authors: *T. W. CLEWORTH[1], T. INGLIS[1,2,3], M. G. CARPENTER[1,2,3];
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Abstract: BACKGROUND AND AIM: Threat-related postural changes, such as decreased amplitude of sway [1,2], occur in conjunction with significant increases in vestibular and proprioceptive gain [3,4]. Recently, this increased sensory gain with threat has been thought to compensate for decreased postural sway, which leads to a discrepancy between perceived and actual sway during quiet stance [5]. The impact these changes in perceived sway have on more voluntary, large amplitude postural movements is currently unknown. Therefore, the aim of this study was to examine how changes in threat influence conscious perceptions of voluntary leaning to different degrees of maximum voluntary lean.
METHODS: Eight young healthy adults, fitted with kinematic markers placed on the right side of the body, stood with eyes open on a forceplate mounted to a hydraulic lift placed at two heights (0.8m and 3.2m). Subjects first stood in the high condition and leaned as far forward as possible, rotating only at the ankle joint, to establish a performance-based limit of stability (maximum lean). Then, at each height, subjects voluntarily swayed to a target (10% to 100%, in increments of 10%, of maximum lean) displayed visually on a screen in front of them as Center of Pressure (COP) or whole body angular displacement. Once the target was reached, subjects reported the amount of perceived lean (as a percent of maximum lean). Psycho-social questionnaires were used to record balance confidence, fear and anxiety. Physiological arousal was recorded from electrodermal activity of the hand (EDA).
RESULTS: Threat significantly increased EDA, fear and anxiety, and decreased balance confidence ($p<0.05$). Subjects were able to voluntary lean to all target positions, based on COP and angular displacement feedback, at both heights equally. However, the amount of perceived lean was greater at High versus Low heights, particularly for targets between 30% and 100% maximum lean.
CONCLUSIONS: When subjects are asked to lean to a target in a threatened state, they perceived themselves to be at a further proportion of maximum lean. These results could be

explained by a fear related increase in sensory gain, and/or a decrease in perceived limit of stability. Discrepancies between actual, and perceived, postural abilities may be an important contributor to increased fall risk in fearful individuals.
REFERENCES: [1] Carpenter et al. (1999) J Vestib Res; [2] Davis et al. (2009) Gait Posture; [3] Naranjo et al. (2015) Neurosci; [4] Horslen et al. (2013) J Neurophysiol; [5] Cleworth et al. (2016) Neurosci Lett.
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Disclosures: T.W. Cleworth: None. **T. Inglis:** None. **M.G. Carpenter:** None.

Poster

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Program#/Poster#: 440.16/YY4

Topic: E.06. Posture and Gait

Title: Postural control as a function of sloped support surfaces

Authors: *A. DUTT-MAZUMDER;
Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: This investigation examined the effects of slope of the surface of support (35°, 30º, 20º, 10º Facing Down, 0° Flat and 10º, 20°, 25° Facing Up) and postural orientation on the margins of postural stability in quiet standing of young adults. The findings showed that the center of pressure - CoP (displacement, area and length) had least motion at the baseline (0° Flat) platform condition that progressively increased as a function of platform angle in both facing up and down directions. The virtual time to contact (VTC) dynamics revealed that the temporal margins to the functional stability boundary were progressively smaller and the VTC time series also more regular (SampEn - Sample Entropy) as slope angle increased. Surface slope induces a restricted stability region with lower dimension VTC dynamics that is further constrained when postural orientation is facing down the slope. Thus, the study shows preliminary evidence of VTC being used as a promising tool to study spatio-temporal control for a postural system across sloped surface.

Disclosures: A. Dutt-Mazumder: A. Employment/Salary (full or part-time): Medical University of South Carolina.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 440.17/YY5

Topic: E.06. Posture and Gait

Support: NIH NCRR Grant P20 RR016435

Title: Influence of task-related and person-related variables on the impaired anticipatory postural adjustments of people with low back pain: insight into heterogeneous results across studies

Authors: *J. V. JACOBS[1], J. R. HITT[2], S. M. HENRY[3];
[1]Ctr. for Physical Ergonomics, Liberty Mutual Res. Inst. for Safety, Hopkinton, MA; [2]Univ. of Vermont, Burlington, VT; [3]Univ. of Vermont Med. Ctr., Burlington, VT

Abstract: Although some studies on the anticipatory postural adjustment (APA) demonstrate the APA is delayed in subjects with low back pain (LBP), other studies have not replicated this finding. Inter-study differences in task-related and person-related factors may account for these differential findings in the literature. Thus, the association of such factors with APA onset latencies must be determined in people with LBP. This study evaluated 13 subjects with LBP and 13 subjects without LBP during seated, rapid arm flexion in self-initiated and cued movement conditions. We evaluated (a) APA onset latencies of the erector spinae, internal oblique, and external oblique muscles relative to deltoid muscle onset with surface electromyography, (b) velocity of arm movement with passive-marker motion capture, and (c) pre-movement cortical negativity with electroencephalography. Task- and person-related factors of movement condition, movement velocity, cortical function, numeric pain ratings, pain-related fear of movement (Fear Avoidance Beliefs Questionnaire; FABQ), and disability level (Modified Oswestry Questionnaire) were therefore evaluated for association with LBP-related changes in APA onset latencies. Results demonstrated a significant group-by-condition interaction, such that APA onset at the external oblique muscle exhibited greater delay for the subjects with LBP compared to the subjects without LBP in the cued condition than in the self-initiated condition. A group main effect demonstrated larger cortical pre-movement negativity for the group with LBP, but also significantly slower arm-raise velocities. When applying arm-raise velocity as a covariate, the interaction effect on the APA latency of the external oblique muscle remained significant, but the group main effect on cortical pre-movement negativity did not. Correlation analysis demonstrated significant correlations of the external oblique muscle's APA latency with Oswestry and FABQ scores in both the self-initiated and cued conditions. These results demonstrate that delayed APAs with LBP can be influenced by the task-related factor of cueing as well as person-related factors of pain-related disability and fear of activity. Differences in pre-movement cortical function appear related to modulating the arm-raise velocity, but delayed

APAs remained evident in the group with LBP independent of differences in arm-raise velocity. These results highlight the importance of subject-sample characteristics and task condition when comparing across studies of postural coordination with LBP.

Disclosures: **J.V. Jacobs:** A. Employment/Salary (full or part-time): Liberty Mutual Insurance. **J.R. Hitt:** None. **S.M. Henry:** None.

Poster

440. Posture: Muscle Activity, Exercise, and Biomechanics

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Program#/Poster#: 440.18/YY6

Topic: E.06. Posture and Gait

Support: American Occupational Therapy Foundation Pilot Grant

College of Health and Human Sciences mini-grant

Title: Smartphone-based assessment of changes in postural stability in neurological patients after a therapeutic exercise intervention

Authors: ***B. L. TRACY**[1], A. A. SCHMID[2], D. M. MILLER[3], K. E. TIMROTH[3], B. E. HOLLAND[3], L. R. JANKOWSKI[3], M. F. FRITZ[3];
[1]Hlth. & Exerc Sci., [2]Occup. Therapy, [3]Hlth. and Exercise Sci., Colorado State Univ., Fort Collins, CO

Abstract: Accurate measures of postural stability provide information on functionally important feature of neuromuscular output in health and disease. Force platforms are accurate and commonly used, but they are expensive, require trained personnel, and are not portable. The modern smartphone, however, contains very sensitive triaxial accelerometers. Apps can log the acceleration values at up to 100Hz, thus the smartphone can serve as a portable, easily used movement sensor in settings outside the laboratory. The purpose was to validate the iPod Touch ($200) against a force platform ($10k) to assess changes in postural stability in neurological patients (stroke, peripheral neuropathy) after an individualized, rehabilitative yoga intervention. Stroke survivors and peripheral neuropathy patients (N=24, 52-92 yrs) were tested before and after eight weeks of supervised yoga. Standing postural stability was measured with the eyes open and closed. Foot stance width was set at 10% of height. The iPod was fixed to a strap just lateral to the greater femoral trochanter. During the 60s trials, the center-of-pressure (COP) underneath the feet and the acceleration at the hip were simultaneously recorded. The two streams of data were time-aligned with a small tap. The standard deviation (SD) of the COP in

A/P and M/L directions and the SD of the acceleration in the corresponding X and Z axis of the iPod was measured to indicate the average variability in the signal. The average of two trials was taken for each visual condition. Change scores were computed for the SD value from pre- to post-training. Correlations were computed between the change values from the two devices for both directions and visual conditions. The change scores were distributed around zero. The change values from the force platform and the iPod were consistently positively correlated. In the M/L direction, the R-squared value was 0.753 for eyes open and 0.538 for eyes closed. In the A/P direction, the R-squared was 0.77 for eyes open and 0.847 for eyes closed. For the total sway values (summed M/L and A/P), the R-squared value was 0.882 for the eyes open condition and 0.781 for eyes closed. When values for eyes open and eyes closed were pooled, the R-squared for the total sway was 0.860. Small changes in postural sway after intervention in neurological patients can be detected reasonably accurately with inexpensive, portable, user-friendly, smartphone technology. These devices may thus provide a suitably sensitive quantitative tool for balance measurement in clinical settings and remote community locations.

Disclosures: **B.L. Tracy:** None. **A.A. Schmid:** None. **D.M. Miller:** None. **K.E. Timroth:** None. **B.E. Holland:** None. **L.R. Jankowski:** None. **M.F. Fritz:** None.

Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.01/YY7

Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant 4R33AI101504

Title: Neuronal-specific cargo-delivery platform for post-botulism therapies

Authors: *V. PATEL[1], M. PIRES-ALVES[2], A. M. PATEL[1], M. HO[2], J. J. MCARDLE[1], B. A. WILSON[2];
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Abstract: Botulinum neurotoxin A (BoNT/A) consists of heavy (Hc) and light (Lc) chains linked by a disulfide bond. Hc targets gangliosides whose proximity to motor nerve terminal active zones facilitates BoNT/A endocytosis by the vesicle membrane recycling process. Lc is a Zn-endoprotease, which acts intracellularly to specifically cleave the SNARE complex protein SNAP-25, and thereby suppress excitation-secretion coupling and produce flaccid muscle paralysis. While in vitro inhibitors of the Lc endoprotease exist, none facilitate functional

recovery for muscles paralyzed in vivo. This is attributed to failure of Lc inhibitors to gain access to the nerve terminal cytosol. To translate in vitro Lc inhibition into therapeutic efficacy, we are investigating fusion proteins (FP) consisting of Hc linked to an inhibitory cargo against Lc. As a proof of concept we examined delivery of GFP into nerve tissue by a FP consisting of Hc linked to GFP. First, we confirmed that the GFP cargo is delivered into the cytosol of cultured NG108-15 cells. Next, delivery of the GFP into motor nerve terminals of mouse Triangularis sterni nerve muscle preparations (NMPs) was examined. NMPs were incubated for 90 min in physiologic solution containing 8-10 µg of FP. Induction of FP endocytosis was induced by depolarization with 40 mM KCl or 5-Hz supramaximal nerve stimulation for 90 min. NMPs were washed and stained with rhodamine-conjugated α-bungarotoxin for 30 min to visualize endplates prior to visualization of neuromuscular junctions (NMJs) using confocal microscopy. Analysis of 25-µm thick optical sections indicated GFP uptake in response to both KCl depolarization and nerve stimulation. Pretreatment of stimulated (5 Hz, 90 min) NMPs with 6.7 pM BoNT/A also accumulated GFP within nerve terminals upon subsequent injection with FP. To determine if GFP is delivered in BoNT/A-paralyzed muscles in vivo, we first injected a mouse hind limb with 3 µl 6.7 pM BoNT/A. The EDL muscle of the injected hind limb was paralyzed within 24-36 hrs. The paralyzed limb was then injected with 10 µl of ~1 mg FP/ml on each of three consecutive days. Mice were then sacrificed, and EDLs were harvested and examined with confocal microscopy. Our observations demonstrate that this novel cargo delivery platform can deliver GFP to control and BoNT/A-poisoned motor nerve terminals. We are now examining various BoNT/A Lc inhibitory cargos for therapeutic efficacy in mice poisoned with this potent neurotoxin.

Disclosures: **V. Patel:** None. **M. Pires-Alves:** None. **A.M. Patel:** None. **M. Ho:** None. **J.J. McArdle:** None. **B.A. Wilson:** None.

Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.02/YY8

Topic: E.10. Motor Neurons and Muscle

Title: Botulinum neurotoxin type A-cleaved SNAP25 is confined to primary motor neurons and expressed on the plasma membrane following intramuscular toxin injection

Authors: ***R. S. BROIDE**, J. FRANCIS, B. B. CAI;
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Abstract: Botulinum neurotoxin type A (BoNT/A) is used clinically for a growing number of therapeutic and cosmetic indications. The present study was performed to assess the potential for neuronal transport and transcytosis of BoNT/A (onabotulinumtoxinA) following peripheral administration. In this study, the expression of BoNT/A-cleaved substrate ($SNAP25_{197}$) was characterized in a rat motor neuron pathway following toxin intramuscular injections at relatively low (3 U/kg) and high (10 & 30 U/kg) doses. Using a highly selective anti-$SNAP25_{197}$ antibody in combination with quantitative, high-resolution 3D imaging, we performed a systematic evaluation to determine whether $SNAP25_{197}$ is confined to the primary motor neurons (MN), or is also found in neighboring cells and/or nerve fibers within the spinal cord (SC). The results demonstrated that $SNAP25_{197}$-IR staining is co-localized with biomarkers for MNs, but is not co-localized with markers for neighboring neurons, nerve fibers or glial cells. Additionally, we found that a high dose of BoNT/A (30 U/kg), but not a lower dose (3 U/kg), resulted in spread of toxin activity to distal muscles, giving rise to sporadic $SNAP25_{197}$ signal in the muscles and associated SC regions without evidence for transcytotic (cell to cell) migration. Despite the spread in $SNAP25_{197}$-IR staining, functional effects, as measured by the Digit Abduction Score assay were not detected in the distal muscles. These results suggest that BoNT/A efficacy is confined to the primary MNs and that any evidence of distal activity is the result of limited systemic spread of the toxin at high doses and not through transcytosis within the SC. Lastly, we discovered that at the higher doses of BoNT/A, $SNAP25_{197}$-IR staining was expressed throughout the MN and was highly co-localized with synaptic markers on the plasma membrane, persisting for at least 6-days following toxin treatment. These final data support previous studies suggesting that $SNAP25_{197}$ acts as a dominant-negative SNARE protein within the affected MNs.

Disclosures: **R.S. Broide:** A. Employment/Salary (full or part-time): Allergan PLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allergan PLC. **J. Francis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allergan PLC. **B.B. Cai:** A. Employment/Salary (full or part-time): Allergan PLC.

Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: Joint Science and Technology Office, Medical S & T Division (CBM.THRTOX.01.10.RC.021)

NIAID (AOD12058-0001-0000)

Title: Pharmacological modulators of presynaptic calcium concentration reverse botulinum neurotoxin-induced silencing of synapses *In vitro* and delay paralysis in *Ex vivo* muscle preparations

Authors: *A. B. BRADFORD, P. H. BESKE, J. B. MACHAMER, T. M. RUSSO, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def, Gunpowder, MD

Abstract: Botulinum neurotoxins (BoNTs) are potent paralytics that cause respiratory arrest through blockade of cholinergic neurotransmission. There are currently no effective treatments to counteract toxin function within the nerve terminal. We have previously characterized 3,4-diaminopyridine (3,4-DAP) as a potential treatment to delay paralysis from multiple BoNT serotypes (BoNT/A, /B and /E) or to assist recovery from partial paralysis. These studies suggested that rescue of neuromuscular transmission in intoxicated nerve:muscle preparations by 3,4-DAP acts through increase release probability. Based on these findings, we hypothesized that increasing presynaptic calcium concentration ($[Ca^{2+}]_i$) enhances presynaptic release of neurotransmitter in intoxicated motor neurons by increasing release probability. To test this hypothesis and to identify potential therapeutic interventions, we performed a primary screen in synaptically-active neuron cultures of candidate drugs that have previously been shown to enhance presynaptic $[Ca^{2+}]_i$ through diverse mechanisms. We identified several compounds that restored spontaneous synaptic activity in cultures intoxicated with BoNT/A. Lead candidates were then tested in an *ex vivo* model of botulism using phrenic nerve-hemidiaphragm (PND) muscle preparations. A subset of these drugs transiently enhanced evoked muscle twitch in paralyzing tissues, delaying but not preventing full-paralysis. Co-administration with 3,4-DAP resulted in additive or synergistic effects on muscle twitch amplitudes, suggesting multiple sources of increased $[Ca^{2+}]_i$ can combine for added effect. Evoked end plate potentials (EPPs) and spontaneous miniature EPPs (mEPPs) were characterized for some of these drugs in PNDs, confirming their mechanism of action involves enhanced presynaptic release probabilities. Collectively, these experiments show the potential of presynaptic therapeutic intervention, indicate that increased $[Ca^{2+}]_i$ delays and temporarily reverses BoNT-induced paralysis, and identify several drugs that offer potential clinical utility in botulism patients.

Disclosures: A.B. Bradford: None. **P.H. Beske:** None. **J.B. Machamer:** None. **T.M. Russo:** None. **P.M. McNutt:** None.

Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: DTRA: CBM.THRTOX.01.10.RC.021

NIAID: AOD12058-0001-0000

Title: 3,4-DAP reverses botulinum-induced muscle paralysis by increasing neurotransmitter release probability at unintoxicated release sites

Authors: *J. MACHAMER, A. BRADFORD, T. RUSSO, P. MCNUTT;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Botulinum neurotoxins (BoNTs) are a potent family of protein-based toxins that inhibit neurotransmission by cleaving and inactivating soluble NSF attachment protein receptor (SNARE) proteins, causing progressive muscle paralysis and death by asphyxiation. There is currently no treatment to accelerate the recovery of neuromuscular function in patients exposed to BoNT. While administration of aminopyridines such as 3,4-DAP have been proposed to reverse symptoms of BoNT paralysis, their mechanism of action is unclear and diverse studies have resulted in mixed conclusions about their efficacy. Here, we use *ex vivo* mouse hemidiaphragm preparations to rigorously evaluate the effects of 3,4-DAP treatment on BoNT intoxication of neuromuscular junctions. Application of 3,4-DAP enhanced the force of muscle contraction and prolonged the time-to-full paralysis in hemidiaphragms intoxicated with human pathogenic BoNT serotypes A, B, or E. Potentiation of contraction strength was inversely proportional to the extent of paralysis, with near-complete loss of contraction enhancement in fully paralyzed preparations. To more precisely evaluate the effects of 3,4-DAP treatment on synaptic function, we recorded evoked postsynaptic endplate potentials in unintoxicated and intoxicated hemidiaphragm muscle cells in the presence or absence of 3,4-DAP. These results indicated that 3,4-DAP works by enhancing release probability at intact (e.g., non-intoxicated) release sites, and therefore the reduced effect size of 3,4-DAP at late stages of disease are the consequence of the decreasing availability of intact release sites. Based on these findings, we modeled the effects of 3,4-DAP on the probability of exceeding threshold neurotransmitter release as the number of intact release sites decreases, representing the progressive intoxication of a single nerve terminal. Collectively, these studies identify the probability of release at intact release sites as a potential therapeutic target for treatment of botulism and identify disease states that are likely to be amenable to 3,4-DAP treatment.

Disclosures: **J. Machamer:** None. **A. Bradford:** None. **T. Russo:** None. **P. McNutt:** None.

Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.05/YY11

Topic: E.10. Motor Neurons and Muscle

Support: Mary Tucker Currie Professorship to JWG

Title: Conditioning at the neuromuscular junction: Elucidating the eliciting conditions

Authors: *M. M. STRAIN, J. D. TURTLE, Y.-J. HUANG, J. W. GRAU;
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Abstract: Research shows that the spinal cord is capable of learning without brain input (Grau, 2014, *Neurobiol Learn Mem*). In these studies, rats with a spinal cord transection were given shock to the tibialis anterior [TA] muscle whenever the hindleg was extended. Rats trained in this manner exhibit a progressive increase in flexion duration. The spinal cord was shown to be necessary for learning this response (Crown, 2002, *Physiol Behav*). However, recent work shows that the spinal cord is not necessary for the maintenance of the memory. Indeed, rats given instrumental training prior to receiving a sciatic transection or intrathecal lidocaine were able to perform the instrumental response. EMG and immunohistochemical evidence indicates that the memory is maintained within the neuromuscular junction (NMJ) (Hoy, 2011, *TAMU library*). Further research has show that the isolated NMJ is capable of Hebbian LTD (Dan, 1992, *Science*). Here we explore the role of the NMJ in learning and maintaining the instrumental response. We began by determining whether learning could be induced at the NMJ by substituting spinal cord input with stimulation of the peroneal nerve. In Experiment 1, rats received a spinal cord and sciatic nerve transection. Twenty-four hours later, rats were prepared for instrumental learning and stimulation of the peroneal nerve. Groups received contingent simulation with either paired stimulation of the nerve and TA or stimulation to the TA or nerve alone. Only rats in the paired condition exhibited an increase in leg flexion. For Experiment 2, we examined whether the response is necessary for learning or if pairing muscle and nerve stimulation was sufficient. Twenty-four hours after the spinal cord and sciatic transection, rats received stimulation to the peroneal nerve that was either paired or explicitly unpaired with TA stimulation. Results indicate that pairing nerve stimulation with TA activation causes a lasting (30 min) increase in flexion. In Experiment 3, we manipulated the timing of the stimulations. Rats were set up as explained above, however instead of simultaneous pairings of the nerve and TA, they received TA stimulation immediately followed by nerve stimulation or nerve stimulation followed by TA stimulation. We found TA activation prior to nerve stimulation produced the largest increase in leg flexion. These studies indicate that the NMJ is sensitive to temporal relations between stimuli and undergoes a form of plasticity. This may have

implications for therapies that rely upon electrical stimulation of muscles to foster locomotor recovery or prevent spasticity. Future studies will examine the underlying mechanisms responsible for NMJ plasticity.

Disclosures: M.M. Strain: None. **J.D. Turtle:** None. **Y. Huang:** None. **J.W. Grau:** None.

Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: DFG Grant JA 1823/2-1

Title: Ighmbp2 deficiency corresponds to protein translation changes and leads to differentiation defects in motoneurons

Authors: *V. SURREY[1], M. MORADI[1], B. DOMBERT[1], H. VYAS[2], C. WINKLER[2], U. FISCHER[3], S. JABLONKA[1];
[1]Inst. for Clin. Neurobio., Univ. Hosp. Wuerzburg, Wuerzburg, Germany; [2]Dept. of Biol. Sci., Natl. Univ. of Singapore, Singapore, Singapore; [3]Dept. of Biochem., Univ. Wuerzburg, Würzburg, Germany

Abstract: Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a monogenic motoneuron disorder caused by mutations in the IGHMBP2 gene encoding for a ribsosome associated ATPase/Helicase. Despite detailed knowledge about underlying genetic alterations, the cellular mechanisms leading to this disease are not well understood. In the neuromuscular degeneration *(Nmd2J)* mouse, which is the mouse model for the juvenile form of SMARD1, similar pathological features such as diaphragmatic palsy and skeletal muscle atrophy as displayed in SMARD1 patients are observed. Ex vivo studies of *Nmd2J* mice revealed that motor axon loss precedes atrophy of the gastrocnemius muscle and does not correlate with neurotransmission failures at the motor endplate. The already described independently occurring myogenic abnormalities in diaphragm and heart of the *Nmd2J* mouse raised the question whether spinal motoneuron degeneration develops cell autonomously. In order to address this question we performed in vivo studies from Ighmbp2 deficient zebrafish embryos, and in vitro studies from Ighmbp2 deficient mouse embryos. In both animal models a markedly enhanced axonal branching in primary motoneurons had been observed. A time lapse imaging study via fluorescence recovery after photobleaching (FRAP) visualized a local translation delay of β-actin mRNA in growth cones and cell bodies from enriched Ighmbp2 deficient mouse motoneurons,

although the amount of β-actin mRNA showed no aberration. All together these data suggest that motoneuron pathology under Ighmbp2 deficiency occurred cell autonomously in association to local protein translation mechanisms.

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Poster

441. Motor Neuron-Muscle Interface

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PR Science & Technology Trust

Title: Effects of tamoxifen on single muscle fiber function and protein expression after spinal cord injury

Authors: I. K. SALGADO VILLANUEVA[1], A. E. RODRIGUEZ[1], A. I. TORRADO[1], M. E. SANTIAGO[1], W. R. FRONTERA[2], *J. D. MIRANDA[1];
[1]Univ. Puerto Rico Sch. Med., San Juan, PR; [2]Physical Med. and Rehabil., Vanderbilt Univ. Sch. of Medicine,, Nashville, TN

Abstract: Spinal cord injury (SCI) is associated with skeletal muscle atrophy and dysfunction. Efforts to treat SCI have focused almost exclusively on restoring nerve communication with little attention to muscle deterioration. The timeline of event leading to muscle changes must be understood in order to develop effective interventions in the acute phase of SCI. Therefore, the purpose of this study was to determine when changes in muscle fiber contractility and biochemical composition start to occur after SCI. We measured the contractile properties of permeabilized single muscle fibers from rat tibialis muscle at 2 and 7 days post-injury (DPI). Female rats were injured at the T10 level with the NYU impactor. SCI resulted in changes in the maximum shortening velocity of single muscle fibers at 7 DPI but no changes were observed at 2

DPI. The expression of type 2B myosin heavy chain was also decreased by 7 DPI. Available treatments for muscle dysfunction have not been shown to be practical nor effective in acute SCI. Tamoxifen (TAM), an FDA approved for the treatment of cancer, is a selective estrogen receptor modulator (SERM) that interacts with estrogen receptors. We treated rats with TAM immediately after SCI and analyzed the expression profile of the contractile protein of soleus, myosin heavy chain-1A (MHC-1A), and the transcription factor associated with satellite cell proliferation in skeletal muscle, Pax-7. Western Blot analysis demonstrated that SCI reduced the amount of MHC-1A in the soleus muscle at 28 DPI and this change returned to basal levels if the injured rats were treated with TAM. Moreover, TAM increased the expression of Pax-7 at 28 DPI in the soleus muscle of rats with SCI. Together, these data suggest that damage to hindlimb skeletal muscles start to show-up within the first week after SCI and a pharmacological agent like TAM may be effective in maintaining the contractile properties of skeletal muscle fibers after trauma.

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Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant AR056330

Gift from the Kahlert Foundation

Title: Association of small ankyrin 1 with sarcolipin

Authors: *A. LABUZA, P. F. DESMOND, J. MURIEL, M. L. MARKWARDT, M. A. RIZZO, R. J. BLOCH;
Physiol., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Small Ankyrin 1 (sAnk1) is a ~20kDa transmembrane (TM) protein that binds to the cytoskeletal protein, obscurin, and stabilizes the network sarcoplasmic reticulum (nSR) in skeletal muscle. We have recently shown that sAnk1 binds to the sarco(endo)plasmic reticulum Ca^{2+}-ATPase 1 (SERCA1) and regulates its activity (Desmond, 2015). Here, we show that sAnk1 binds to a second regulator of SERCA1, sarcolipin (SLN). Antibodies to sAnk1 co-immunoprecipitate (co-IP) with SLN in COS7 cells transfected to express both proteins, as well

as from isolated SR membranes. An anisotropy-based FRET method (AFRET) also demonstrated the close association of sAnk1 with SLN. Close association was further demonstrated by the use of half-Venus constructs of sAnk1 and SLN. The C-terminal portion of Venus was fused to SLN while the N-terminal portion was fused to sAnk1. Independently, neither show fluorescence, but when co-expressed these two constructs were fluorescent, indicating reconstitution of Venus. We now propose to test the possibility that a three-way complex exists containing sAnk1, SLN, and SERCA1, by using the two half-venus constructs and a cerulean form of SERCA in AFRET assays. The likelihood that a 3-way complex forms is suggested by the fact that antibodies to SERCA1 co-IP more sAnk1 from transfected COS7 cells when SLN is present than when it is absent. We anticipate that AFRET will confirm this.
In preliminary studies, we also show sAnk1, SLN, and SERCA are all expressed within the brain, suggesting that SERCA activity may be regulated similarly in neurons as in muscle. These results have significant implications for the development of therapeutic approaches to treat a variety of diseases linked to calcium misregulation such as muscular dystrophies and neuropathies.

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Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.09/ZZ1

Topic: E.10. Motor Neurons and Muscle

Title: Oxidative stress and reduced gsh:gssg ratio in ryr1-related myopathies.

Authors: ***M. S. RAZAQYAR**, J. WITHERSPOON, J. ELLIOTT, I. ARVESON, K. MEILLEUR;
NINR, Bethesda, MD

Abstract: RYR1-Related Myopathies (RYR1-RM) are a group of disorders that are caused by mutations in the skeletal muscle ryanodine receptor gene *RYR1*. Mutations in *RYR1* hamper RyR1's ability to regulate calcium and cause either RYR1-RM or Malignant Hyperthermia. RYR1-RM comprise the most common group of congenital onset myopathies, affecting over 1/90,000 people in the United States. It is also the second most common group of muscle diseases in childhood. Individuals present with symptoms of generalized hypotonia, muscle weakness, delayed motor milestones, fatigability, and, in severe cases ophthalmoplegia, scoliosis, and respiratory failure. RyR1 calcium dysregulation leads to oxidative stress, impaired

excitation-contraction coupling, and characteristic skeletal muscle fiber type predominance. A recent study by Dowling *et al.* (2012) conducted in a zebrafish model of RYR1-RM and human myotubes implicated mitochondrial oxidative stress as an important pathophysiological mechanism in RYR1-RM. Durham et al. (2008) also showed reduced levels of an endogenous antioxidant, GSH, in a mouse model of RYR1-RM. GSH counteracts oxidative stress by being oxidized into glutathione disulfide (GSSG). Both groups separately showed that N-acetylcysteine (NAC), a precursor of GSH, reduced oxidative stress in all three preclinical models. Because preclinical models of RYR1-RM have shown increased mitochondrial oxidative stress and decreased GSH, we hypothesized that the GSH:GSSG ratio would be decreased in RYR1-RM patients compared to healthy controls.Whole blood was collected from 27 fasting patients (14 adults, 13 children), immediately mixed with agents for preservation of thiols, centrifuged, and frozen. Analysis to determine plasma GSH:GSSG ratios was performed at Emory as previously described (Jones et al. 2009). The mean GSH:GSSG ratio for the 27 patients was 13.2 ($\pm$ 5.84) with an adult mean of 14.3 ($\pm$ 5.05) and a pediatric mean of 12.1 ($\pm$ 6.61). The difference in ratio of GSH:GSSG between adult and children was not significant. The previously reported mean for healthy adults was 21.3 $\pm$ (10.5). There was a significant difference in mean GSH:GSSG ratio between RYR1-RM adults and healthy adults (p=0.016). This is the first report of GSH:GSSG ratios being reduced in patients with RYR1-RM, suggesting increased oxidative stress in this population. Mutations in *RYR1* hamper RyR1's ability to regulate calcium leading to mitochondrial oxidative stress, among other comorbidities. We are conducting a double blind, placebo-controlled trial in RYR1-RM patients to determine NAC's effectiveness in reduction of oxidative stress and alleviation of associated symptoms.

Disclosures: M.S. Razaqyar: None. **J. Witherspoon:** None. **J. Elliott:** None. **I. Arveson:** None. **K. Meilleur:** None.

Poster

441. Motor Neuron-Muscle Interface

Location: Halls B-H

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Program#/Poster#: 441.10/ZZ2

Topic: E.10. Motor Neurons and Muscle

Support: Canadian Foundation for Innovation, Grant #32952

Title: Functional analysis of $Na_v1.4$ mutation in a case presenting with Schwartz-Jampel features, myotonic discharges, and herculean development

Authors: X. XIONG[1], D. H. FELDMAN[1], T. L. KLASSEN[2], C. G. SANCHEZ ACOSTA[3], L. PLAZA-BENHUMEA[4], R. MASELLI[1], *C. LOSSIN[5];

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Abstract: Schwartz-Jampel syndrome (SJS) is an autosomal recessive skeletal dysplasia characterized by varying degrees of myotonia and chondrodysplasia linked to mutations in the heparin sulfate proteoglycan perlecan gene, *HSPG2*. The details of the underlying molecular pathophysiology of this syndrome are poorly understood. We report on a case with SJS-like features including myotonic discharges in EMG and herculean musculature; both parents were asymptomatic. Whole-exome sequencing revealed no relevant genetic alterations in HSPG2, but it did identify a variant in the skeletal muscle voltage-gated sodium channel $Na_v1.4$ gene, *SCN4A*, which has well-established ties to myotonic disorders. The associated amino acid substitution falls into a highly conserved region of $Na_v1.4$, which is predicted (SIFT) to have significant impact on the function of the channel. To obtain a better understanding of the mutant channel's biophysical abnormalities, we conducted whole-cell voltage clamping and found subtle changes in the voltage dependence of in-/activation and fast inactivation recovery. Slow inactivation was affected as well, in that the mutant tended to require shorter, less depolarizing pulses to enter slow inactivation, and longer hyperpolarization to recover from the same. Preliminary analysis suggests a net loss-of-function, which is in agreement with the recessive pattern of transmission of this pedigree, but it constitutes a challenge when it comes to explaining the myotonic component of the disorder. Furthermore, the absence of genetic alteration in *HSPG2* and the lack of an obvious connection between $Na_v1.4$ dysfunction and chondrodysplasia opens the possibility of another, so-far unknown contributing factor, pointing to genetic heterogeneity in SJS.

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Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.11/ZZ3

Topic: E.10. Motor Neurons and Muscle

Support: Ontario Graduate Scholarship

NSERC Grant

Title: Chronic inflammatory demyelinating polyneuropathy weakness is associated with reduced muscle mass and motor unit loss

Authors: *K. GILMORE[1], K. KIMPINSKI[2], T. DOHERTY[3], C. RICE[4];
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Abstract: Introduction: Chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired neuropathy of immunological origin. It is characterized, usually, by symmetrical weakness in proximal and distal muscles that progresses for greater than 2 months. Evidence indicates that disease-related post-synaptic damage and muscle fiber atrophy lead to neuromuscular junction remodeling and impaired neuromuscular transmission (Deschenes et al. 2011). Increased understanding of the functional limitations and pathophysiology of CIDP are of particular interest due to the treatable nature of the disease. We sought to compare dorsiflexion strength, motor unit (MU) numbers of the tibialis anterior (TA) and TA cross sectional area (CSA) in CIDP (n=6) with age (64 ±15) and sex matched controls (n=6).
Methods: The TA was selected due to its known involvement in CIDP and its high-degree of accessibility for needle EMG. In an isometric dynamometer, dorsiflexion strength with twitch interpolation to assess voluntary activation was recorded. Surface and concentric needle electromyographic (EMG) signals were collected from the TA . To estimate the number of MUs in the TA the decomposition enhanced spike-triggered averaging technique was applied (Stalberg and Sonoo, 1994). This required subjects to contract isometrically at 25% of their maximal voluntary strength for a series of 30s contractions until at least 20 suitable MU trains were acquired. MRIs were acquired via serial axial planes in a 3.0 Telsa magnet. Proton density images for anatomical measures were acquired using a 2D FLASH sequence with the following parameters: 1,500 ms repetition time (TR), 14 ms echo time (TE), 256×192 matrix, 243×325 mm field of view, 30 slices, 5-mm slice thickness with slice separation of 2 mm. OsiriX (v 7.0, 32 bit) MR imaging software was used to measure maximal TA muscle CSA.
Results: CIDP patients were ~50% weaker than controls despite near maximal voluntary activation (~98%) of the dorsiflexors. The estimated number of functioning MUs in the TA was ~45% fewer in CIDP patients. MRI results indicated that CIDP patients had ~55% less TA muscle area compared to controls. From these preliminary results, those with CIDP have significantly less dorsiflexion strength likely due to fewer numbers of MUs innervating the TA and substantially lower TA muscle mass as reflected by smaller muscle CSA compared with controls.
Conclusions: Dorsiflexion muscle weakness in CIDP patients appears multifactorial and is due to loss of MUs, which may be directly related to muscle atrophy of the TA.

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Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.12/ZZ4

Topic: E.10. Motor Neurons and Muscle

Support: COST 015/13 (COST Action FA1301)

EP7 STIFF-FLOP project

Title: Biophysical and structural properties that contribute to the special biomechanics of the octopus arm

Authors: *L. ZULLO[1], F. MAIOLE[2], S. M. FOSSATI[3], N. NESHER[4], B. HOCHNER[5]; [1]Inst. Italiano Di Tecnologia, NSYN, Genova, Italy; [2]Univ. of Genova and Inst. Italiano di Tecnologia, NSYN, Genova, Italy; [3]NSYN, Inst. Italiano di Tecnologia, Genova, Italy; [4]Sch. of Marine Sci., The Ruppin Academic Ctr., Michmoret, Israel; [5]Dept. Neurobiology, Silberman Inst. of Life Sci., The Hebrew Univ., Jerusalem, Israel

Abstract: The *Octopus vulgaris* arm is a highly flexible structure with a virtually unlimited number of degrees of freedom. The arm's extraordinary motor capabilities are achieved despite the absence of a rigid skeleton and a composition of mainly incompressible muscle tissues. In this study we aim at elucidating the structural and biophysical properties that contribute to the special biomechanical properties of the octopus arm musculature and their neuromuscular control. Especially we want to investigate a new hypothesis whereby in the octopus the muscle and collagen tissue close interactions contribute to create stiffening not only due to the antagonistic action of the different muscle groups, but also due to "particles jamming" possibly resulting from stiffening-induced increase in friction between arm's muscle cells and connective tissue. In order to do so we analyzed passive and active force/velocity relationship in both isometric and isotonic activation of arm musculature segments. For this we use a Dual-Mode Lever Arm System on in-vitro preparations. We found that each muscle type might differently contribute to the arm stiffening and that their coordinated action might be the key element for the formation of jamming forces in the arm. We then asked how the neuromuscular system is organized to control this special biomechanics. We performed a confocal microscopy investigation of muscles by using specific staining markers for presynaptic nerve terminals and postsynaptic acetylcholine (ACh) receptors. We found that ACh receptors are concentrated in a signal 'eye shaped' invagination (≈5X20μm) along the cell longitudinal axis. Motor neurons terminals were also localized in a single spot in close opposition with the ACh receptors. Although these results suggest a direct neural control of each muscle cells, it is still possible that a system of gap junctions between the muscle cells serves to control and coordinate the basal level of muscle responsiveness. As the presence of Innexins (the main constituents of

invertebrate gap junctions) might be a good indicator for gap junction-like structure, we cloned part of the octopus innexin gene and evaluated its expression at the level of various tissues. Real Time PCR analysis revealed that innexin is differentially expressed in tissues such as muscle, brain and skin. Next, we will perform in-situ hybridizations to determine its tissue distribution. Taken together these findings have an important relevance in the understanding coordination control mechanisms and biomechanics of this muscle hydrostatic limb.

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Poster

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Topic: E.10. Motor Neurons and Muscle

Support: J.R. Whitaker was supported by a master's fellowship from the SIUC Graduate School.

Title: Light-activation of channelrhodopsin-2 expressed in chick embryo hindlimb muscle mimics neural activation of muscle

Authors: ***J. R. WHITAKER**[1], S. FROMHERZ[2], P. R. PATRYLO[1], A. A. SHARP[3]; [1]Physiol., Southern Illinois Univ. Carbondale, Carbondale, IL; [2]Biol., Saginaw Valley State Univ., University Center, MI; [3]Anat., Southern Illinois Univ. Sch. of Med., Carbondale, IL

Abstract: Numerous studies have shown that proper neuronal and muscle activity is required for normal development of central motor circuitry as well as peripheral structures such as the neuromuscular junction. The elucidation of how each component of this complex system contributes to the normal process of development is generally hampered by the non-selective nature of pharmacological agents. Accordingly, our goal was to directly and non-invasively control excitation of select groups of muscle fibers in a living chick embryo in order to better understand the role of muscle activity in neuromuscular development. Previously, we developed an optogenetic approach that allowed persistent heterologous expression of a fusion protein consisting of a channelrhodopsin-2 variant (ChIEF) and the fluorescent reporter tdTomato (Tom) in neural tissue of living chick embryos (Sharp & Fromherz 2011). In order to drive expression of ChIEF-Tom in muscle tissue, we replaced the CAG (chicken β-actin) promoter upstream of the ChIEF-Tom coding region in our original expression plasmid with the muscle-specific, myosin light chain promoter (Wang et al. 2011). Electroporation was used to deliver plasmid DNA encoding ChIEF-Tom to hindlimb muscle precursor cells in the somites of embryonic day

(E) 3 chick embryos. Following electroporation, fluorescence of the Tomato reporter was detected in muscle fibers of embryos between E6 and E18. Histological examination of chick hindlimbs revealed muscle fiber specific expression of ChIEF localized to the plasma membrane and distinguishable from immunohistochemically-labeled myosin heavy chain in the cytoplasm. To test for functionality, the hindlimbs of chick embryos were carefully exposed *in ovo* on E9 or E10 and light from a blue LED was directed onto hindlimb muscles showing ChIEF expression. Light-evoked movements were quantified using video-microscopy and kinematic analysis. Light-activation of ChIEF resulted in hindlimb movements corresponding with the muscles that showed ChIEF expression. Muscle contraction persisted for the duration of light exposure (seconds) without decrement. Both the rate and magnitude of evoked movements were directly related to changes in light intensity and duration. Bursts of short duration (5-10 ms) light pulses could be used to imitate neuronal activation of muscle. For example, adjustment of pulse frequency and duration altered movement amplitude and rate and both fused and unfused tetanus could be obtained. Our findings indicate that light-activation of ChIEF expressed in chick hindlimb muscle can be used to better understand the role of muscle activity in neuromuscular development.

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Poster

441. Motor Neuron-Muscle Interface

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Program#/Poster#: 441.14/ZZ6

Topic: E.10. Motor Neurons and Muscle

Title: Development of a separation method of neural component from spastic resistance

Authors: *K. TAKEDA[1], S. MIZUNO[2], H. MAEDA[2], K. OHNO[1], A. ORAND[1], G. TANINO[1], H. MIYASAKA[1], S. SONODA[2];
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Abstract: Spasticity is involuntary muscle tone which causes resistance to motion. Quantification of increased joint resistance by the abnormal neural activity during passive rotation is important in stroke rehabilitation. Clinical evaluation of spasticity is usually performed by modified Ashworth scale (MAS) which is an ordinal scale that assesses the resistance during passive joint movement by the examiner. Recent studies, including ours, developed some devices which quantitatively measure the resistance torque during manually or

motor-driven passive movement because the MAS has been criticized for their low reproducibility. Although the quantitative measurement of resistance torque is meaningful, the observed torque includes not only neural but also non-neural components. Therefore, in the present study, we propose a new method to divide the resistance torque into neural component and viscoelasticity. A custom made device, electromotor installed ankle-foot orthosis, was used to rotate an ankle joint of stroke patients with spasticity. The resistance torque (plantar flexion torque), angle, and surface electromyography (sEMG) of the gastrocnemius (GC) and tibialis anterior (TA) muscles were simultaneously measured at 1 kHz sampling from 20 degrees of plantar flexion to over 10 degrees of dorsiflexion. We measured the resistance torque at two different angular velocity; approximately 5 deg/s (low velocity) and 90 deg/s (high velocity). The sEMG signals were rectified and integrated via a time window of 400 ms. For the resistance torque at the low velocity condition, a three-piece linear regression was applied to evaluate the coefficients of elastic element which depend on the muscle length (joint angle). The elastic element is common by the resistance torque of low velocity and high velocity conditions. Viscous component, which depends on the angular velocity, and neural components based on the sEMG signals of GC and TA with time delay were separated by using a system identification approach from the elasticity subtracted resistance torque at the high velocity condition. Then, the plantar flexion torque of ankle joint which formed by passive rotation could be divided into elastic, viscous, GC neural, and TA neural components with less residual error.

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Poster

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Program#/Poster#: 441.15/ZZ7

Topic: E.10. Motor Neurons and Muscle

Title: Wireless innervation of neurons using electromagnetic fields in craw

Authors: *B. KUTER[1], E. C. GUSTAFSON[3], E. P. WIERTELAK, 55105[2];
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Abstract: Transcranial magnetic stimulation (TMS) has been shown effective in the treatment of a variety of post-stroke movement disorders. That TMS has this effect may indicate some underlying neuronal mechanism that can be modulated by TMS' electromagnetic fields (EMF). As an initial approach to examining such a possibility, the effect of TMS was examined by

exposing standard crawfish electrophysiology preparations to varying strengths of EMFs generated by a dual metal-plate array. The crawfish were prepared by dissecting off their tails, and exposing their superficial flexor. This muscle was then probed with a microelectrode, and placed between the two EMF-generating plates. The inserted microelectrode then recorded the resting membrane potentials of the muscle. Signals ranging from 2.0 MHz to 5.0 MHz were tested, in increments of 0.5 MHz with exposure and resting times of 35 seconds. When the mean difference between each frequency on and off levels were compared, the application of EMF at all strengths produced significant effects on The results indicated that unknown neuronal mechanisms were being affected by the EMFs. There was an optimal frequency range present in the tested frequencies around 4.5 MHz. Future research in this laboratory will focus on examination of effects in this range. Elucidation of the underlying mechanism for this effect of EMF could help shed light on the processes involved in EMF-exposure, and in the further future lead to methodology on reliably controlling a cell's resting membrane potential wirelessly.

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Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: NSF STC EBICS CBET 0939511

NSF IGERT NeuroEng 0903622

Title: A three-dimensionally engineered spinal cord-skeletal muscle bioactuator

Authors: *C. S. LIU[1], C. CVETKOVIC[2], G. NASERI KOUZEHGARANI[3], R. BASHIR[2], M. U. GILLETTE[3];
[1]Cell and Developmental Biol., [2]Bioengineering, [3]Neurosci., Univ. of Illinois, Urbana, IL

Abstract: We have successfully created an *in vitro* 3D bioactuator using rat spinal cord tissue and C2C12 skeletal muscle cells. This is a major step towards enabling the fabrication of biological machines capable of sensing, processing the input, and exerting a force upon the surrounding environment. Whereas, previous studies have successfully created neuromuscular junctions in static 2D cultures (Das *et al, Biomaterials* 2010), here we show dynamic activation of culture muscle strips due to neuronal input on a recently developed 3D platform (Cvetkovic *et al*, *PNAS* 2014, Raman *et al*, *PNAS* 2016, Chan *et a*l, *Scientific Reports* 2012). Skeletal muscle

strips were created on a 3D printed flexible skeleton in order to engineer a muscle actuator. Spinal cord sections taken from the postnatal day 3-5 rat pups were then placed onto the muscle strip and allowed to grow in a 50/50 mixed spinal cord and muscle differentiation medium. The spinal cord tissue adhered well to the muscle strip and sent out substantial extensions into the surrounding myotube bundles. Using immunohistochemistry, we examined the efficacy of neurite outgrowths from the spinal cord and subsequent innervation into the muscle strips, leading to neuromuscular junction formation. We also examined functionality of the 3D engineered biological machine using glutamate stimulation to specifically excite neurons of the spinal cord and patch-clamp electrophysiology to demonstrate robust electrical activity and health in the nascent biological machine. This system realizes the goal of creating a functional, biomimetic machine with neuronal input that is capable of movement through the normal physiological mechanisms of the neuromuscular junction. This achievement advances us along toward the eventual goal of realizing a forward-engineered, autonomous integrated cellular machine and system with a host of applications for tissue engineering, drug testing, and a better *in vitro* model for studying development of nervous and musculoskeletal systems.

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Poster

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Program#/Poster#: 441.17/ZZ9

Topic: E.10. Motor Neurons and Muscle

Title: The effect of an 8 week CrossFit type exercise program on inflammatory injury and balance

Authors: *G. S. BAINS[1], E. LOHMAN[1], L. BERK[1], N. DAHER[1], R. CHETTIAR[1], O. AMBODE[1], B. MIRANDA[1], R. SINGH[1], F. NUGENT[2];
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Abstract: CrossFit type exercises (CFE) are a combination of resistance and aerobic training which involve constantly varied intensity and functional movements. High sensitivity C Reactive Protein (hs-CRP) is a known inflammatory biomarker. To our knowledge, there have been no published studies showing the relationship of CrossFit exercises on balance improvement and on hs-CRP modulation. **PURPOSE:** To evaluate the effect of 8 weeks of CFE on inflammation through acute hs-CRP modulation and balance parameter measurements compared to a no

exercise control group. **METHODS:** Sixteen adult subjects were randomly assigned to a CFE group (25.7 ± 2.2 years, n=9) or a control group (25.6 ± 3.1 years, n=7). Previously, all subjects were exercising at a low level of intensity for a minimum of 6 weeks and were not trained in CFE. The CFE gradually increased in intensity weeks 1 to 4, and then increased to a very heavy intensity weeks 4 to 8. High sensitivity CRP concentrations were drawn at baseline and 48 hours post exercise at the end of weeks 1,4,8, and 9. Additionally, vertical jump height and balance testing (limits of stability) were assessed at baseline and at 48 hours post exercise at week 8. **RESULTS:** There was no significant change in hs-CRP concentrations over time within each group. For hs-CRP concentrations for the CFE group, there was a significant change ($p=.04$) from baseline to week 1 (23.1%), a borderline significant change ($p=.07$) from week 1 to week 9 (21.1%), and no significant change ($p=.73$) from baseline to week 9 (1.4%). Additionally, from baseline to week 8, vertical jump height, reaction time, and movement velocity each significantly increased ($p<.05$) in the CFE group by 13.2%, 21.2%, and 30.7% respectively. There was no significant change in hs-CRP, vertical jump height, reaction time, and movement velocity in the control group. **CONCLUSION:** We suggest, after completing 8 weeks of CFE program, subjects were observed to have a reduced inflammatory response as assessed by hs-CRP. Although we continuously increased exercise intensity throughout the study, hs-CRP concentrations did not significantly change from baseline to week 9. However, we suggest that the CFE group was able to gradually acclimate to the CFE program. Therefore, this modulation in inflammatory response could be suggestive of the resultant acclimation that translates for an improvement in vertical height jump level and faster reaction time and movement velocity in mainstream CrossFit athletes. CFE is an effective training program in maintaining a minimal non-significant inflammatory response as assessed by hs-CRP 48 hours post exercise. Further research is suggested to expand these encouraging findings.

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Poster

441. Motor Neuron-Muscle Interface

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Topic: D.03. Somatosensation: Touch

Support: NSERC PGS-D to GEP

NSERC Discovery Grant to EPZ

NSERC PGS-M to SN

Title: Intermittent leg cycling sprints induce fatigue-related suppression of human soleus Hoffmann reflexes

Authors: *G. E. PEARCEY[1], S. NOBLE[1], B. MUNRO[2], J. L. BISHOP[2], E. ZEHR[1]; [1]Rehabil. Neurosci. Lab., Univ. of Victoria, Victoria, BC, Canada; [2]Exploration Team, Nike Sport Res. Lab., Beaverton, OR

Abstract: Intermittent sprints (IS) are known to induce neuromuscular fatigue that can be attributed to a combination of central and peripheral mechanisms. Furthermore, fatigue due to tetanic stimulation of the triceps surae induces suppression of the soleus Hoffmann (H-) reflex, as does non-fatiguing arm and/or leg cycling. However, it is unknown whether cycling IS alter the excitability of the soleus 1a monosynaptic reflex. Thus, the purpose of this experiment was to determine if fatiguing IS alter the excitability of the soleus H-reflex. 13 healthy young participants performed 7 x 10 s sprints interleaved with 3 min unloaded cycling on a Monark ergometer. Sprints were braked with 9% mechanical loading and participants were instructed to cycle all-out for the 10s duration. H-reflex and M-waves of the right soleus were evoked by stimulation of the tibial nerve at the popliteal fossa. M-H recruitment curves were recorded pre- and post-session at rest. H-reflexes corresponding to ~90% maximal H-reflex amplitude were evoked during unloaded cycling just prior to the first IS, after each sprint and then 5 and 10 minutes after sprint 7. Results indicated that there was significant fatigue throughout the IS protocol, since the average power output was reduced by 6.2 ± 2.29% ($p = 0.036$) by sprint 3 and remained reduced by up to 14.2 ± 2.52% ($p = 0.001$) during sprint 7. Following sprints 1 through 7, H-reflex amplitudes were suppressed by 22.5 - 30.5% ($p < 0.01$), and remained suppressed by 21.7 ± 2.52% ($p = 0.001$) 5 min following sprint 7. Pre- to post-protocol comparisons of resting M-H recruitment curves revealed that there was an increase in the minimum current required to evoke an H-reflex (threshold) and a decrease in the maximal H-reflex amplitude. These data suggest that IS induce significant fatigue that results in reduced power output, and the fatiguing effects of IS result in decreased excitability of the human lumbar spinal cord.

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Poster

441. Motor Neuron-Muscle Interface

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Topic: E.10. Motor Neurons and Muscle

Support: PAPIIT-UNAM IN212916 (MMG)

CONACYT 417840 (RL)

Title: Bladder and urethral function in female rabbit: a model of damage

Authors: *R. LOPEZ JUAREZ[1,2], R. ZEMPOALTECA[3], D. CORONA-QUINTANILLA[3], F. CASTELÁN[4,3], M. MARTÍNEZ-GÓMEZ[4,3];
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Abstract: In female mammals, the lower urogenital tract is essential for excretory and reproductive functions including micturition, defecation, sexual intercourse and crossing of fetuses during deliveries. Pelvic and perineal striated muscles play important roles in the physiology of the lower urogenital tract. In micturition, the pelvic muscles participate in urine storage, whereas the perineal muscles participate in voiding of urine. We reported that that blocking the activity of perineal muscle bulbospongiosus (Bsm), ischiocavernosus (Iscm) and pelvic muscle as the pubococcygeus (Pcm) causes changes in micturition similar that multiparous female rabbits. So a model of damage was done to the nerves of the pelvic and perineal muscles. Virgin female chinchilla rabbits 10 to 12 months old were used in the study. Cystometrograms were recorded simultaneously with electromyograms and intraurethral pressure, with and without denervation and/or crushing of Bsm was evaluated. Pcm electromyographic activity was recorded as control. After denervation of Bsm the urodynamic parameters showed decrease in voiding volume and increased in residual volume. In intraurethral pressure, decreased the maximum pressure and threshold pressure. When the nerve of Bsm was crushed, the threshold pressure and the maximum pressure decreased and the voiding duration increased. The intraurethral pressure decreased and the intraurethral threshold increased. Electromyographic records shown activity of Bsm during the expulsion and activity of Pcm during urine storage. Bsm activity disappeared after denervation, and after crushing, the activity showed diminished and disorganized. The results suggest that the Bsm contributes to the regulation of urine expulsion, and if its innervation is damaged during delivery, could be affect its function.

Disclosures: **R. Lopez Juarez:** None. **R. Zempoalteca:** None. **D. Corona-Quintanilla:** None. **F. Castelán:** None. **M. Martínez-Gómez:** None.

Poster

441. Motor Neuron-Muscle Interface

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 441.20/ZZ12

Topic: E.10. Motor Neurons and Muscle

Title: Identifying the function of tyramine in the mouse uterus

Authors: M. AGRE, B. OBAYOMI, L. TOWNLEY, *D. P. BALUCH;
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Abstract: Pregnancy and the birthing process are natural events but still little is known about the signaling mechanism(s) that induce contractions. Globally, premature labor occurs in 12% of all pregnancies resulting in 15 million babies born preterm. Even though the cause of preterm labor can vary, understanding the signaling pathway that regulates muscle contraction could provide additional treatment options to stop premature labor. The uterus is composed of smooth muscle and in conjunction with the associated nerve fibers, forms a plexus which covers the muscle fibers. The plexus has swollen areas called varicosities that contain neurotransmitters. Within the uterine tissue, the smooth muscle receives opposing inputs from the sympathetic and parasympathetic parts of the ANS. Smooth muscle can be stimulated or modulated by many sources such as neurotransmitters (i.e. norepinephrine), hormones (i.e. epinephrine) and chemicals (i.e. nitrous oxide). In this study we are researching an alternative modulator of smooth muscle activity, a monoamine produced in the catecholamine biosynthesis pathway called tyramine. During catecholamine biosynthesis, dopamine, tyramine, octopamine, and norepinephrine are all derived from the tyrosine precursor. Tyramine is known to be associated with peripheral vasoconstriction, increased cardiac output, increased respiration, elevated blood glucose and release of norepinephrine. Our research has found tyramine and its specific receptor TAAR1 to be localized at the uterine neuromuscular junction in relation to muscular contraction. This project is focused on distinguishing the synchronous and alternating signaling pathways, between the epinephrine and octopamine pathways, which regulate muscle contraction.

Disclosures: **M. Agre:** None. **B. Obayomi:** None. **L. Townley:** None. **D.P. Baluch:** None.

Poster

441. Motor Neuron-Muscle Interface

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 441.21/ZZ13

Topic: E.06. Posture and Gait

Title: New measurement system of human arm stiffness for clinical evaluation

Authors: *T. YUKAKO[1], K. IGARASHI[1], S. KATSURA[1], F. NAKAI[2], C. YAMADA[2], Y. ITAGUCHI[3], H. YOSHIZAWA[4], K. FUKUZAWA[2];
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Abstract: The present study reports a new robotic-measurement system of arm stiffness for clinical evaluation. Quantitative evaluation using robotic devices has advantages to implement efficient rehabilitation programs. To assess sensorimotor abilities of upper limb, arm stiffness is considered as one of integrative indices. Arm stiffness is defined as an end-point force of an arm generated in response to an applied displacement. Arm stiffness at a certain position can be represented as an ellipsoid. It helps us to understand musculoskeletal condition of an arm including muscle rigidity and abnormal reflex.Conventional systems for arm stiffness measurement required a parallel-link-driven manipulandum consisting of rotational motors, the size of which was too large to install in clinical settings. They used a force sensor to measure arm stiffness, which could deteriorate measurement accuracy. A gantry robot developed in our laboratory was composed of three linear-direct-drive motors, which reduced the size of the device and realized high-accuracy of measurement due to exclusion of coordinate transformation. Further, the robotic device can measure arm stiffness in three dimensions, which might be useful in clinical situations.We estimated stiffness ellipsoid based on measured arm stiffness using the robotic device. Six students participated in the experiment (four males and two females, from 21 to 25 years old). Their upper and lower arms were hanged to eliminate gravity effects. They gripped a vertical handle attached to the device during the measurement. Perturbations were applied to the participants' arm through the handle in 26 directions (one trial). The amplitude of perturbations were 8mm and lasted 850ms with a 150ms ramp. We used force and position data for 150ms from the onset of perturbation to estimate arm stiffness. Arm stiffness was measured three times at one location and at five different locations in total. The time for one trial took about 100s. A stiffness ellipsoid was calculated by multiplying the stiffness matrix and the unit sphere. Stiffness matrix was derived using a least mean square method. The results showed that major axes of the ellipsoids were oriented to the shoulder of the participants, which is the prominent property of arm stiffness. We observed the same range of stiffness values compared to the previous studies, which validated our measurement system. In

addition, the variance of the measurement was small (about 63 % in SD of stiffness matrices). The equivalent stiffness values and small variance in measurement suggested that one trial is adequate for daily use of our system in clinical settings.

Disclosures: **T. Yukako:** None. **K. Igarashi:** None. **S. Katsura:** None. **F. Nakai:** None. **C. Yamada:** None. **Y. Itaguchi:** None. **H. Yoshizawa:** None. **K. Fukuzawa:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Program#/Poster#: 442.01/ZZ14

Topic: F.01. Neuroethology

Support: NSF Grant CRCNS-IIS-1208118

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Title: Rat whiskers are used in airflow sensing

Authors: ***Y. S. YU**[1], M. M. GRAFF[1], C. S. BRESEE[2], Y. B. MAN[1], M. J. Z. HARTMANN[1,3]; [1]Dept. of Mechanical Engineering, Northwestern Univ., Northwestern Univ. Dept. of Mechanical Engin., Evanston, IL; [2]Northwestern Univ. Interdepartmental Neurosci. Program, Evanston, IL; [3]Northwestern Univ. Dept. of Biomed. Engin., Evanston, IL

Abstract: Mechanisms for flow sensing and anemotaxis are well studied in arthropods, and in some aquatic and flying mammals. Remarkably, however, no study to date has yet investigated the sensory cues used by terrestrial mammals for anemotaxis. The present work is the first to investigate the cues that allow terrestrial mammals to detect and localize airflow.Two lines of evidence suggest that rat vibrissae can be used for flow sensing. First, the mechanical response of a rat vibrissa to airflow contains information about both flow direction and magnitude(*1*). Second, a common neural drive of whisking and sniffing(*2*) confers a degree of temporal precision that could align anemotaxic information with odorant information during olfactory search. We therefore tested the hypothesis that the vibrissae contribute significantly to the ability of rats to localize airflow. In this study, five rats trained on a five-alternative forced-choice airflow localization task exhibited significant performance decrements after vibrissal removal. In contrast, vibrissal removal did not disrupt performance of control animals trained to localize a

light source. Importantly, the performance decrement of individual rats was correlated with their airspeed threshold for successful localization; animals that found the task more challenging relied more on the vibrissae for localization cues. Following vibrissal removal rats deviated more from the straight-line path to the air source, choosing sources further from the correct location. Results demonstrate that the rodent vibrissal-trigeminal system, which has a well-established role in tactile detection and texture discrimination, also contributes significantly to anemotaxis. This discovery provides a link between the vibrissotrigeminal system and olfactory search, and offers a behavioral rationale for the coupling between oscillatory neural circuits that drive sniffing and whisking. In the field of ethology, our results enable comparisons of flow sensing behaviors in terrestrial mammals, arthropods, and pinnipeds, and could inform analysis of foraging behavior.
References: (1) Yu YSW, Graff MM, Hartmann MJZ. Mechanical responses of rat vibrissae to airflow. *J Exp Biol* 219, 937-948 (2016). (2) Moore JD, Deschenes M, Furuta T, Huber D, Smear MC, Demers M, Kleinfeld D. Hierarchy of orofacial rhythms revealed through whisking and breathing. *Nature* 497, 205-210 (2013).

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Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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NSF EFRI-0938007

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NDSEG 32 CFR 168a

Title: The three-dimensional morphology of vibrissal follicles and muscles: implications for motor control

Authors: *C. S. BRESEE[1], J. L. ALADE'FA[2], L. A. HUET[3], H. BELLI[2], M. J. Z. HARTMANN[4];
[1]Interdepartmental Neurosci. Program, [2]Dept. of Biomed. Engin., [3]Dept. of Mechanical Engin., [4]Dept. of Biomed. Engineering, and Dept. of Mechanical Engin., Northwestern Univ., Evanston, IL

Abstract: Rats use active, rhythmic movements of their mystacial vibrissae (whiskers) to tactually explore the environment. Recent work has suggested that rats can change tactile sensing resolution by modifying the "spread" or the "spacing" of the whiskers. Spread is defined as the angular distance between the rostral- and caudal-most whiskers on one side of the face, while spacing is defined as the distance between adjacent whiskers. In the present work, we quantified aspects of the mystacial pad anatomy that underlie the rat's ability to change spacing and spread. We focused on the geometry of the whisker follicles and the intrinsic muscles that connect two adjacent follicles within a row. We sectioned four complete mystacial pads, performed three-dimensional reconstructions of all follicles, and identified the attachment points of the intrinsic muscles around the follicles. Results showed that all whiskers emerge from the mystacial pad at approximately equal angles relative to the skin. Follicle length and spacing, as well as muscle attachment points, all vary in proportion to each other across the array. Simulations based on these measurements suggest that if each intrinsic muscle were to receive the same rate of activation from lower motor neurons, all whiskers would rotate approximately through the same angle. In other words, a motor command sent to any lower motor neuron projecting to an intrinsic muscle could be in a common rate code. Thus the geometry of the pad ensures an invariance at the level of lower motor neurons, leaving changes in sensing resolution under voluntary control at more central levels.

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Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

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Program#/Poster#: 442.03/AAA2

Topic: F.01. Neuroethology

Support: The East Carolina University Division of Research and Graduate Studies fund to F.I.

Title: The effect of social stress on the dopaminergic pathway of the escape circuit in zebrafish

Authors: *K. CLEMENTS[1], T. MILLER[1], E. JI[2], F. ISSA[1,2];
[1]Biol., East Carolina Univ., Greenville, NC; [2]Physiol., UCLA, Los Angeles, CA

Abstract: The occurrence of a chronic stressor causes an elongated state of physiological changes, affecting both peripheral and central body systems in a detrimental manner to the health of the organism. One approach to test the effects of a chronic stress is to induce a social hierarchy. Zebrafish form social hierarchies that consist of either socially dominant or subordinate fish. We have observed that once a social hierarchy has been established, behavior patterns between males reflect their social standing; social dominants display aggressive behaviors (attacks), while socially subordinates display submissive behaviors (retreats). The objective of this project is to determine the effects of chronic stress on dopaminergic neurotransmission and its effects on the highly characterized Mauthner escape circuit. When startled, zebrafish produce a stereotyped escape response that is mediated by auditory activation of the Mauthner command neurons which innervate spinal motor neurons. We tested the effects of social stress on the Mauthner escape response by recording the far-field potentials of fish escape to auditory pulses of increasing intensity (70-105 decibels). We observed that socially subordinate fish have a lower threshold for producing an escape when compared to both dominant and communal fish. These results suggest that a chronic state of stress on the subordinates influences the underlying neural signaling responsible for this escape behavior. To better understand the neural bases of social status-dependent change in Mauthner sensitivity, we tested whether social stress affects the dopaminergic system. Through application of the dopamine precursor, L-DOPA, we have found that the dopaminergic system is socially regulated. We have also applied dopamine receptor agonists and antagonists. We observed that chronic stress may be affecting the presence of dopamine 1 (D1) receptor in submissive animals, causing a sensitization in production of escape response. Gaining a better understanding of how chronic social stress influences the dopaminergic pathway will facilitate the advancement of new treatments of disorders that disrupt natural dopaminergic function.

Disclosures: K. Clements: None. **T. Miller:** None. **E. Ji:** None. **F. Issa:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Program#/Poster#: 442.04/AAA3

Topic: F.01. Neuroethology

Support: East Carolina University division of Research and Graduate Studies fund

Title: Social status-dependent molecular regulation of dopaminergic pathways in the brain of zebrafish (danio rerio)

Authors: *T. H. MILLER[1], K. CLEMENTS[1], E. JI[3], F. ISSA[2];
[1]Biol., [2]East Carolina Univ., Greenville, NC; [3]Univ. of California, Los Angeles, CA

Abstract: In zebrafish (*Danio rerio*), social interactions between adult males consist of a series of aggressive encounters that ultimately lead to the formation of stable hierarchies of either socially dominant or subordinate animals. Although it has been shown that social status leads to neurophysiological changes in brain structure and function, our understanding of how identified brain circuits are modulated by social status in vertebrate model systems is limited. Preliminary results have shown that there is a social-status dependent effect on the sensitivity of the C-start escape response. We hypothesize that the activation pattern of the Mauthner neural circuit, that mediates the C-start escape response in zebrafish, is likely affected by social experience through the regulation of the dopaminergic system. The focus of this study is to determine how social experience affects the regulation of the expression on genes in the dopaminergic pathway in the zebrafish brain, and how this may relate to the Mauthner neural circuit. Our results indicate that the brain-wide dopaminergic system is modulated on a transcriptional level, with social status-dependent regulation of dopamine supply and receptor expression. We show that although there were no significant differences in the expression of tyrosine hydroxylase (th), dopa decarboxylase (ddc) and vesicular monoamine transporter (vmat) in dominant and subordinate animals, we found that whole brain expression of dopamine active transporter (dat) was significantly up-regulated in dominant animals compared to subordinates. In addition, drd1b receptor expression was down-regulated in dominants compared to subordinates. Finally, the hypothalamic and hindbrain sub-regions also display social status-dependent transcriptional modulation of the dopaminergic system. To fine tune our examination of the dopaminergic system relative to the Mauthner Neural circuit, we plan to examine expression of dopaminergic genes in the Mauthner Command neurons and specific regions of the hypothalamic nuclei known to project to the spinal cord. Our findings suggest that there is a social-status dependent regulation of the dopaminergic system via modulation of pre- and post- dopaminergic synaptic pathways.

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Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Topic: F.01. Neuroethology

Support: (NSF-IOS) 1456830

Title: Trade-offs between speed and variability in responses to looming visual stimuli

Authors: *K. D. BHATTACHARYYA[1], D. L. MCLEAN[2], M. A. MACIVER[2];
[1]Biomed. Engin., [2]Northwestern Univ., Evanston, IL

Abstract: Predation of mobile animals by other mobile animals, in which the biological arms race between predator and prey can led to increasing morphological and neural complexity, may have been the central mechanism for the Cambrian radiation. Two of the weapons prey have for survival in predatory encounters are 1) reducing the predictability of their escape maneuver and 2) increasing the speed of their escape maneuver. However, high accelerations and velocities are subject to biomechanical constraints and working at the limit of biomechanical capacity may vitiate variability. Consequently, animals may need to trade-off speed and variability in escape behaviors - implying that behavioral urgency plays a role in the variability of an escape maneuver. A time-sensitive aversive stimulus, like the looming stimulus, which is the rapid expansion of an image creating an impression of an approaching object or predator, can be used to interrogate the impact of behavioral urgency by tuning the rate of approach or image expansion. We investigated whether the variability of the escape trajectory was inversely related to the rate of expansion or approach of the looming stimulus by studying the angle of escape in the looming-evoked responses of larval zebrafish. In a free swimming assay, we found that variability in the angle of escape does indeed increase with a more gradual expansion of the looming object. Additionally, in a partially-restrained preparation, we performed calcium imaging of the Mauthner cell, a command neuron known to be involved in escape turns, with simultaneous high-speed imaging of body kinematics during escape responses to looming stimuli. We discovered that rapidly approaching stimuli are more likely to activate the Mauthner cell. The kinematics of escape responses mediated by Mauthner cell activity were more stereotyped than Mauthner cell inactive responses that are more common with slowly looming stimuli. The results provide insight into a fundamental question in ethology of how animals produce variable or flexible behavior to fixed stimuli.

Disclosures: **K.D. Bhattacharyya:** None. **D.L. McLean:** None. **M.A. MacIver:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Topic: F.01. Neuroethology

Support: NIH Grant EY002686

Mathers Foundation Grant to Jon H Kaas

Title: The visual system of the northern elephant seal

Authors: *E. C. TURNER, E. K. SAWYER, J. H. KAAS;
Vanderbilt Univ., Nashville, TN

Abstract: Northern elephant seals (*Mirounga angustirostris*) are part of a diverse clade of carnivorous animals known as pinnipeds (seals, sea lions, and walruses). Pinnipeds are notable for their large, ape-sized brains, yet little is known about the central nervous system of these federally protected animals. The northern elephant seal spends most of its life at sea, but surfaces briefly on land each year to breed and birth; this unique coastal niche may be reflected in specific evolutionary adaptations to their sensory systems. Here we report on select parts of the visual pathway (optic nerve, lateral geniculate nucleus, and primary visual cortex) of the nervous system of the northern elephant seal. Using stains for Nissl, cytochrome oxidase, and vesicular glutamate transporters in one elephant seal pup and the external anatomy of the brain of a second pup, we investigated the cytoarchitecture of the lateral geniculate nucleus and primary visual cortex. Similar to in rodents and primates, we find that these markers are useful for the identification of architectonic borders. We find that the lateral geniculate nucleus has distinct layers similar to that of closely related species. Primary visual cortex is located within the most posterior end of the highly gyrified cortex, and extends far anterior along the dorsal surface and medial wall. Toluidine blue was used to visualize myelinated axons in the optic nerve of the northern elephant seal, and we found two distinct classes of myelinated fibers, those with thick myelin sheaths (9%) and those with thin myelin sheaths (91%). Axons with thick myelin sheaths tended to be larger in diameter and clustered near each other in one small section on the edge of the optic nerve, while the rest of the optic nerve contained a more consistent distribution of many thinly myelinated axons mixed with fewer thickly myelinated axons. The results may be useful for other comparative studies related to the evolution of large brains.

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Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: NSFC Grant 31301882

Title: Predation behavior is based on background thermoregulation in a pit-viper Gloydius brevicaudus

Authors: *Q. CHEN[1], Y. TANG[2];
[1]Chengdu Inst. of Biology, Chinese Acad. of Sci., Sichuan, China; [2]Chengdu Inst. of Biology, Chinese Acad. of Sci., Chengdu, China

Abstract: Predators with excellent vision are able to distinguish even very small optical differences between target and background. For pit-vipers, the thermal sense is believed to complement vision and provide a substitute imaging system in dark environments. For example, the thermal sense would image a scene consisting of a living mouse in a cold cave as a bright spot on a dark background, projected onto the topographic map of the midbrain tectum. This raises the question of how the thermal imaging system would perform if background temperatures were variable across different portions of the scene. In order to find out we investigated prey capture under such circumstances in the short tailed pit viper, *Gloydius brevicaudus*. Snakes used in this study were tested in a modified predation cage in which the background temperature was maintained at 33℃(normal) on one side of the cage and 40℃ (heated) on the opposite side using a feedback controller. Each of twenty four short-tailed pit vipers was tested on five predation trials. Snakes successfully captured prey on 78 of 120 trials. In the successful trails, snake preferred to strike prey on the normal side (26℃) rather than the warmer side (33℃). Nevertheless testing revealed no orientation bias when they snakes chose between a 26℃and 40℃background temperature difference. In conclusion, the infrared system cannot be viewed as a simple thermal sensor, because increasing the intensity of background radiation does not necessarily mask the target signal. Furthermore the infrared imaging system functions analogously to how the visual system can identify a grey target against a black background as well as a white background as shown by the effect of varying the background temperature from 26℃to 33℃to 40℃. Thus this behavioral research suggests that the pit viper infrared system can accomplish a 'brightness constancy' computation reflecting the difference between the target and the background which is analogous to they way the visual system computes the luminance difference between the target and the background.

Disclosures: **Q. Chen:** None. **Y. Tang:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Topic: F.01. Neuroethology

Support: National Brain Research Centre core grant

Department of Science and Technology SR/CSI/03/2010

Title: A three-dimensional stereotaxic mri brain atlas of house crows (corvus splendens)

Authors: *S. SEN[1], S. PAUL[1], P. RAGHUNATHAN[1], S. S. KUMARAN[2], S. IYENGAR[1]; [1]Systems Neurosci., Natl. Brain Res. Ctr., Gurgaon, India; [2]All India Inst. of Med. Sci., delhi, India

Abstract: The cognitive abilities of the Corvidae family of birds are comparable to those of higher mammals. Higher brain functions such as tool-use, logical deduction, long-term facial memory and the use of working memory which these birds exhibit are almost at par with those of apes and chimpanzees. Amongst corvids, crows are especially proficient at these tasks and fast emerging as a potential model to study these complex cognitive behaviors. Currently, there is an increasing interest in understanding the neural basis of higher cognitive functions across different species using non-invasive neuroimaging modalities such as functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET). MRI-based anatomical brain atlases are used as the standard reference space in neuroimaging studies. In order to decrease the variability between various brain structures or functions and to compare across different experimental animals of the same species, individual MR images are normalized to the brain atlas space. Since there was no such atlas available as a reference for the crow brain, for the first time, we have constructed a three dimensional (3D) MRI brain atlas of the Indian house crow (*Corvus splendens*) using high resolution iso-voxel structural MR images of their brain.The brain atlas encompasses an MRI brain template and a parcellation map delineating major avian brain areas such as the striatum, different areas of the pallium and brainstem. Our brain atlas provides a standard reference space for neuroimaging-based studies and would be useful for marking stereotaxic locations of brain regions at any given head-angle. It would also be valuable for various surgical procedures such as injecting neuroanatomical tracers or pharmacological agents into the brain, electrophysiological recordings as well as brain tissue sectioning. This 3D brain atlas of the house crow will be made freely available on our institutional website.

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Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Program#/Poster#: 442.09/AAA8

Topic: F.01. Neuroethology

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Regressive evolution of the hagfish visual system: blind but hopeful monsters

Authors: *W. T. ALLISON;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: We study the Pacific Hagfish in an evo-devo framework, with special attention to the first appearance of the vertebrate eye over evolutionary time. Other cyclostomes (lampreys) and all jawed vertebrates share the familiar camera-style eye. The last common ancestor of lamprey and vertebrates also had a photoreceptive pineal gland. Thus the Hagfish, oft-reported to lack a pineal, eyes and vision, are positioned perfectly to understand the appearance of the vertebrate eye over evolutionary time. The eyes of adult hagfish are quite small and buried under a layer of epidermis, leading credible sources to suggest hagfish lack vision altogether and their eyes are pineal-like in several characters.

Here we revisit the rarely-examined Pacific Hagfish (*Eptatretus stoutii*), with special attention to the smallest (≈youngest) individuals we can acquire, and applying contemporary histology methods to reveal novel compelling arguments that hagfish possess almost all features of a vertebrate eye; this codifies hagfish eyes as functional, though degenerating during ontogeny. Young hagfish possess a camera-like cup-shaped eye, though we have yet to observe a classical vertebrate lens. We found the ocular media to be broadly transmissive to light from the UV to red portions of the spectrum (340-700 nm). We used a panel of 22 antibodies to perform immunohistochemistry on hagfish eye cryosections. Three independent opsin antibodies all reveal robust photoreceptors at the outer nuclear layer and indistinguishable from vertebrates. Hagfish photoreceptor outer-segments are aligned into an apparent RPE homolog containing engulfed photoreceptor outer segments detected by both electron microscopy and opsin IHC. Importantly, young hagfish retinas have three recognizable nuclear layers separated by synapses as detected by multiple stains and antibodies. Thus, several data sets all independently demonstrate that young hagfish have eyes that are not pineal-like, but have organization at the tissue- and molecular-level shared with lampreys and all jawed vertebrates. Therefore the retina of Pacific Hagfish degenerates with ontogeny and adult tissues have been mis-interpreted to implicate that the eyes of hagfish never develop and never evolved. Further, examination of young hagfish brain reveals a pineal gland, based on specific and polarized opsin IHC labelling at a ventricle wall in the dorsal midline. This serves as a final argument against the need for

hagfish eyes to exclusively serve a pineal-like function. In sum, here we discover and detail hagfish eyes and vision, revising our understanding of the early vertebrate eye evolution.

Disclosures: W.T. Allison: None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

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Program#/Poster#: 442.10/AAA9

Topic: F.01. Neuroethology

Support: Sloan Foundation BR2014-105

Title: Modulation of locomotor behaviors by a dopaminergic population in the zebrafish hypothalamus

Authors: *J. P. BARRIOS[1], A. D. MCPHERSON[3], S. ANJEWIERDEN[2], J. B. NEWTON[2], S. J. LUKS-MORGAN[1], R. I. DORSKY[1], A. D. DOUGLASS[1];
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Abstract: Dopamine (DA) is a key modulator of locomotor networks in multiple contextsIn vertebrates, DA modulates locomotor behaviors at multiple sites, including the spinal cord and the basal ganglia of vertebrates. DA shifts these networks between distinct functional states that optimize sensorimotor processing to best deal with dynamic environmental challenges. A major challenge in neuroscience is to understand the flexibility of sensorimotor circuits imparted by neuromodulators like DA. In this work, we have shown that a specific population of DA neurons in the zebrafish is involved in the generation of locomotor behaviors and we are now investigating the circuit level mechanisms by which this occurs. This DA population is genetically defined by expression of tyrosine hydroxylase-2 (TH2) and is specifically expressed in the hypothalamus. We have shown that ablation of these neurons reduces the frequency of spontaneous locomotor behaviors and optogenetic stimulation increases that frequency. We are currently using a combination of *in-vivo* imaging, ablation, and optogenetic approaches tTo elucidate the circuitry involved inanatomical and functional basis of this locomotor effect., Wwe have begun to rigorously characterize the behaviors evoked by optogenetic stimulation of *th2*+ neurons. Stimulation can evoke both startle and routine swim behaviors, depending on stimulation light intensity. Furthermore, we are using projection-tracing techniques to identify anatomical targets of *th2*+ neurons. We are also using in-vivo two-photon calcium imaging to correlate the activity of *th2*+ neurons with specific spontaneous locomotor behaviors in a head-

fixed, tail-free preparation. Our dataOptogenetic stimulation show that *th2*+ neuron activity of *th2* neurons is sufficient to lower audiomotor startle threshold, further suggesting a role of these neurons in the modulation of startle behavior. We are currently investigating the role of DA from this population in prepulse inhibition of the startle response using chemogenetic ablation and a *th2* knockout zebrafish. Understanding the role of this hypothalamic DA population in the modulation of sensorimotor circuitry in the zebrafish will provide key insights into the flexibility of neural circuits and the implications of that flexibility for behavior.

Disclosures: **J.P. Barrios:** None. **A.D. McPherson:** None. **S. Anjewierden:** None. **J.B. Newton:** None. **S.J. Luks-Morgan:** None. **R.I. Dorsky:** None. **A.D. Douglass:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 442.11/AAA10

Topic: F.01. Neuroethology

Title: Lesions of the telencephalon and the application of a dopamine D1 receptor antagonist result in similar modifications of the acoustic startle response in goldfish.

Authors: *A. N. OPALKA[1,2], N. FISCHER[1], R. F. WALDECK[1,2];
[1]Biol., [2]Neurosci. Program, The Univ. of Scranton, Scranton, PA

Abstract: Normally goldfish make a quick, complete turn away from a vibratory stimulus known as a startle response, which is mediated by the Mauthner cell (M-cell) and connected to motor neurons in the spinal cord. Previous studies have shown that full ablation of the telencephalon significantly decreased the likelihood of a complete startle response in goldfish (Collins & Waldeck, 2006). However, the precise identification of the location within the telencephalon is not known nor the projection to the M-cell in the brainstem. Different regions of the telencephalon were lesioned, and fish were then tested for the resultant behavior to determine the specific location of cells related to modification of the startle response. In a second set of experiments, a dopamine D1 subtype receptor antagonist (SCH23390) was applied to determine dopamine involvement in this modified startle response. Acoustic startle responses were tested and recorded for three consecutive pre- and post-testing days in both sets of experiments. In one set of fish, different areas of the telencephalon were lesioned and then tested for changes in startle response. In a second set of fish, no surgery was performed, but fish were immersed in SCH23390 solution on the fourth day followed by post-testing after immersion for three consecutive days. Behavior analysis was carried out using Logger Pro 3.9 to determine mean-startle angle (MSA). In regards to the location, MSA differs between medial and lateral

telencephalon lobe lesions. Right- and left-medial lesions decrease MSA while right- and left-lateral lesions have less effect. In regards to a possible dopaminergic projection from the telencephalon, statistical analysis revealed significant differences in the MSA between control and antagonist groups. Fish immersed in the D1 antagonist showed similar turning modifications as those fish following lesions of the telencephalon. Future directions hope to show the neuroanatomical projections from the telencephalon to the brain stem.

Disclosures: A.N. Opalka: None. **N. Fischer:** None. **R.F. Waldeck:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 442.12/AAA11

Topic: F.01. Neuroethology

Support: DePauw University

Title: From drug discovery to neural mechanisms of nicotine-induced motor behavior in zebrafish

Authors: *H. SCHNEIDER;
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Abstract: New pharmacotherapeutics for smoking cessation therapy can be identified using zebrafish neurobehavioral assays. Gaining a better understanding of the action of newly discovered chemicals involves the recording of neural network activity in the brain. The goal of this project is to develop chemical treatment schedules that can be used in both nicotine-response assays of freely swimming larval zebrafish and electrophysiological or imaging experiments of embedded or suspended larval zebrafish. Translating experiments on freely swimming zebrafish larvae to fixated or suspended individual larva required certain adjustments of nicotine dosage and application method. Agarose embedding did not seem to change the overall response of larval zebrafish to nicotine, but delayed an increase in locomotor activity by 10 to 20 min compared to the immediate onset in freely swimming larvae. Using lower concentrations, allowed for repeated nicotine applications that alternated with embryo water rinses. High nicotine concentrations have caused irreversible changes in locomotor activity. Focal application of chemicals directly onto the head region reduced the delay that occurs when drugs are applied to the water for transdermal delivery. In recording experiments, focal application onto the head or injections into the brain could minimize adverse effects of chemicals and reduce behavioral changes due to irritant properties of chemicals that could be associated with transdermal

delivery. Developing methods for nicotine-response assays in embedded or suspended larval zebrafish represents the first step towards measuring nicotine-induced neuroadaptations of larval brain activity using methods such as calcium-imaging.

Disclosures: H. Schneider: None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

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Program#/Poster#: 442.13/AAA12

Topic: F.01. Neuroethology

Support: CIHR

NSERC

Title: Processing of sensory input by pyramidal neurons in the electrosensory lateral line lobe of the weakly electric fish Apteronotus albifrons

Authors: *D. MARTINEZ, M. CHACRON;
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Abstract: One of the main goals of neuroscience is to understand how neuronal activity represents different features of sensory input. Studies have shown that the basic building blocks of the brain are common across vertebrates and that higher cognitive abilities result from more complex connectivity patterns. A comparative approach between species is thus likely to uncover general strategies used by the brain to encode sensory input.
Weakly electric fishes are a diverse group composed of many genera. In particular, species within the *Apteronotidae* genus produce a quasi-sinusoidal electric field through the electric organ discharge (EOD). These fish can detect EOD perturbations caused by prey and/or conspecifics through an array of peripheral receptors that then synapse onto pyramidal neurons within the electrosensory lateral line lobe (ELL). Within the *Apteronotidae* genus, species *Apteronotus albifrons* and *Apteronotus leptorhynchus* are closely related. Indeed, both species have very similar body morphology and almost identical brain anatomy (Maler, 2009). Yet, at the same time, both species display important differences in EOD properties as well as in the structure of electrocommunication signals (Kolodziejski, J et al, 2007). On the one hand, one might expect that both *A. leptorhynchus* and *A. albifrons* use similar coding strategies because of their nearly identical brain anatomy. On the other hand, recent studies have shown large heterogeneities in the physiology of neurons with identical morphology. To answer this, we

recorded the spiking responses of pyramidal neurons within the ELL of *A. albifrons* both in the absence and in the presence of electrosensory stimuli. Our stimuli consisted of both natural (sinusoidal) as well as artificial (noise) stimuli in which we varied first and second-order attributes. A comparison between our results and those obtained previously in *A. leptorhynchus* revealed surprising similarity between ELL pyramidal neural responses to similar stimuli. Our results strongly suggest that both *A. albifrons* and *A. leptorhynchus* employ similar coding strategies and that previously observed differences between communication signals and behaviors are used to distinguish between and con and hetero-specifics.

Disclosures: D. Martinez: None. **M. Chacron:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 442.14/AAA13

Topic: F.01. Neuroethology

Support: Sally Mcdonell Barksdale Honors College UM

Title: Dance complexity related to the volume of a sensorimotor region in manakins

Authors: *W. HELMHOUT[1], G. PANO[1], W. R. LINDSAY[2], L. B. DAY[1];
[1]Biol., Univ. of Mississippi, University, MS; [2]Goteborg Univ., Goteborg, Sweden

Abstract: Manakins are a family of birds the males of which use acrobatic, non-vocal display behaviors to attract females to mate. Across the manakin family (Pipridae), species perform displays of varying complexities with variation in the number and type of display sites, acrobatic postures, and number of mechanical sounds. Females of at least one species, select males on the basis of 10s of ms differences in performance of certain male display elements suggesting strong sexual selection. Our lab recently showed a positive relationship between display complexity and brain weight, brain volume, and relative cerebellar volume in manakins suggesting specializations of motor regions might be contributing to species differences in overall brain size. The arcopallium (AP), is another brain region likely to be specialized for complex displays. AP has both motor and limbic functions, and in oscines (songbirds), a specialized portion of the AP, the robust nucleus of the arcopallium (RA), is known to function in song production of vocal courtship displays. Manakins are suboscines that do not appear to have vocal learning or an RA. However, the AP has been shown to be larger in golden-collared manakin males that perform displays, than in females that do not. In addition, the AP in golden-collared manakins contains many androgen receptors, similar to those found in the RA of songbirds; a trait not seen in other

suboscines that do not have complex display. Thus, the AP in manakins is capable of responding to testosterone (T), and because display in manakins in known to be activated by T, the AP could play a role in the courtship behaviors of manakins. Another area, the Nucleus Taeniae (Tn) of the AP, could also be implicated in display complexity. Tn has been shown to have high concentrations of androgen receptors, and though once considered part of AP, it is exclusively limbic and may have distinct function from AP. We compared AP, Tn, and nucleus rotundus (Rt) volume of 12 different manakin species and the closely related ochre-bellied flycatcher; species were chosen for their varying display complexities. The volume of Rt, a visual thalamic nucleus, was used as a control. Marginal means from a general linear model and mean residuals from a least squares regression analysis were used as size adjusted volumes. Adjusted volumes were regressed on complexity using phylogenetic generalized least squares to adjust for relatedness. We found a significant positive relationship between AP volume and display complexity of the manakins' non-vocal courtship behaviors, but no relationship between Tn or Rt and display complexity.

Disclosures: W. Helmhout: None. **G. Pano:** None. **W.R. Lindsay:** None. **L.B. Day:** None.

Poster

442. Neuroethology of Sensory and Motor Systems: Vertebrates

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: NSF Grant 1555916

Title: Olfactory navigation in realistic odor landscapes: modeling and simulation of odor localization and path-following strategies in mice

Authors: *J. HENGENIUS[1], N. URBAN[2], B. ERMENTROUT[3];
[2]Dept. of Neurobio., [3]Dept. of Mathematics, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Navigation via chemical gradients is vital for survival and reproduction in motile organisms, but airflow at the spatial scales of animal navigation is dominated by turbulence. The resulting spatiotemporal fluctuations in odor concentrations prevent animals from relying on direct estimation of local odor gradients for directional cues. Despite these fluctuations, animals are observed using robust strategies to localize odor sources in turbulent environments. We consider possible olfactory navigation strategies in mice, an organism widely used in behavioral and neurobiological study of olfaction. Despite a wealth of information about mouse olfaction, the properties turbulent odor environments and their effects on navigation strategies are poorly

understood. Our approach was two-pronged. First, we measured the statistical properties of experimental odor environments using photo-ionization detection (PID) of the odorant methyl salicylate and light sheet imaging of advected particulates. Second, we characterized rule-based navigation algorithms and their performance in simulated odor landscapes of varying complexity. Measured odors exhibited decreasing mean and increasing coefficient of variation with increasing distance from the source, suggesting mice might use sniff concentration and/or sniff-to-sniff fluctuations in concentration to determine their position relative to a source. We therefore evaluated several model classes: (1) binaral, concentration comparison between nostrils, (2) casting, lateral head movement while sampling concentration, (3) intermittency, evaluating time intervals between odor detection events, and (4) hybrid strategies. All models were simulated in spatial arenas containing odorant point sources or trails, with odor environments of varying levels of realism ranging from noise-free static distributions to turbulent landscapes. All four strategies increased mouse dwell times in high concentration regions of the arena relative to random walks, though only binaral and casting models displayed dynamics qualitatively similar to observed mouse behavior. Interestingly, we found that near-source dwell times were increased by odorant noise in the in binaral and hybrid models, suggesting that animals may exploit turbulence to improve odor localization. Additionally, low-pass filtering (via simulated neuronal computation) improved the concentration signal-to-noise ratio in extremely intermittent odor landscapes. These findings provide insight into the role of turbulent flow in olfactory navigation - not only as a source of noise but as a property that improves localization.

Disclosures: **J. Hengenius:** None. **N. Urban:** None. **B. Ermentrout:** None.

Poster

443. Genetic Approaches in Songbirds

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 443.01/AAA15

Topic: F.01. Neuroethology

Support: NIH T32 MH020065

Title: Bidirectional manipulation of mTOR signaling reveals complicated contribution to sensory learning

Authors: *S. AHMADIANTEHRANI[1], S. E. LONDON[2];
[2]Psychology, Inst. for Mind and Biol., [1]Univ. of Chicago, Chicago, IL

Abstract: The mechanistic target of rapamycin (mTOR) serine/threonine kinase is a nexus in a complex signaling cascade that regulates protein translation in neurons. Disruption of mTOR signaling is implicated in neurodevelopmental and psychiatric disorders, and is routinely demonstrated to affect learning and memory. We previously reported that in juvenile zebra finch songbirds, mTOR signaling is required for tutor song memorization, the sensory learning foundation of learned vocal communication. In adults, sensory song learning is used for individual recognition, essential for establishing and maintaining mate bonds and navigating the colony community. Both tutor song memorization in juveniles and adult song recognition learning require processing within the auditory cortex. Here, we asked if mTOR signaling is also implicated in adult song recognition learning. We verified that major components of the mTOR cascade are present and phosphorylated in the adult auditory cortex after hearing song. We employed an established paradigm of song recognition learning to assess if mTOR activation is modulated: all birds hear 3hr of "training" song playbacks one day, then either no song (Trained-silence), the same song (Trained-familiar), or a different song (Trained-novel) 24hr later. We measured phosphorylation of the ribosomal protein S6 (pS6), which depends on mTOR activity, as a readout for cascade activation. In unmanipulated birds, we see maximal levels of pS6 in the auditory cortex of birds that hear novel song, low levels in Trained-silence, and intermediate levels in Trained-familiar group. This pattern recapitulates the profile of zenk (zif268, egr-1, ngfi-a, krox24) induction. As zenk is a molecular marker for active learning processes, these data support the conclusion that mTOR signaling contributes to adult song recognition learning. Manipulation of mTOR to decouple song experience from signaling, however, revealed unexpected results. We hypothesized that both constitutive activation and inhibition of mTOR signaling during the training experience would disrupt learning and memory events and transform the molecular signature for familiar song into one that mirrored that of novel song. However, when mTOR was constitutively activated, we found pS6 levels equivalent to that in unmanipulated birds; inhibition decreased pS6 levels to those of Trained-silence birds. In combination with control experiments, these results lead us to suggest the relationships between mTOR and learning and memory may be different in juvenile and adult brain, and reconsider the mechanisms by which learning of ethologically-relevant vocal signals may occur.

Disclosures: S. Ahmadiantehrani: None. **S.E. London:** None.

Poster

443. Genetic Approaches in Songbirds

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 443.02/AAA16

Topic: F.01. Neuroethology

Title: An efficient and flexible gene manipulation strategy in zebra finch brain

Authors: *S. E. LONDON[1,2], S. AHMADIANTEHRANI[3];
[1]Dept of Psychology, [2]Inst. for Mind and Biol., [3]Dept of Psychology, Inst. for Mind and Biol., Univ. of Chicago, Chicago, IL

Abstract: The connections between genes, brain, and behavior are fundamental to our understanding of neurobiological processes. Songbird models meaningfully contribute to many fields as the system has great strengths due to its developmental biology, quantifiable behavioral characterization, defined neural circuits for cognition and behavior, sequenced genome, and strong parallels to human speech acquisition. However, likely because of immune system properties, gene delivery strategies commonplace in other systems have been more difficult to implement in songbird brain. We were motivated to develop a reliable, efficient, and flexible strategy to manipulate the genome in brain cells of the songbird in service of directly testing gene-brain-behavior relationships. We therefore adapted in vivo electroporation procedures for use in the early Posthatch zebra finch chick. Briefly, plasmid constructs are infused into the lateral ventricles of Posthatch day 3 (P3) chicks. Paddles placed on the head deliver square voltage pulses that permit plasmids to enter cells along the ventricle. Neuroanatomical specificity is obtained via careful paddle positioning. We use a transposase, also expressed off of a plasmid construct and co-electroporated with the transgene plasmid(s), to integrate transgenes into the genome. We optimized the voltage, duration, and frequency of the pulsing; transposase type; paddle placement; and the age of electroporation. We achieve a high level of survivability (>95%) and efficiency (>60% of ventricular cells express transgenes), stable expression through P50, and neuroanatomical specificity - a large majority (~75%) of transgene-expressing cells were concentrated in the targeted brain region, the auditory cortex. The procedure is effective with multiple constructs co-electroporated. This strategy is therefore appropriate for gene delivery experiments that test circuit and behavioral hypotheses using a variety of manipulations, including gene overexpression or interference including CRISPR editing, inducible technologies, optogenetic or DREADD cellular control, and cell type-specific expression.

Disclosures: S.E. London: None. **S. Ahmadiantehrani:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: QMUL interbal funding

In part by Canadian Institute for Advanced Research

Title: Epigenetics of learning in zebra finch

Authors: *D. CONDLIFFE[1], J. GEORGE[1], C. BARTON[1,2], A. LEITAO[2], M. GAHR[2], P. HURD[1], D. CLAYTON[1];
[1]Sch. of Biol. and Chem. Sci., Queen Mary Univ. of London, London, United Kingdom; [2]Max Planck Inst., Institute for Ornithology, Germany

Abstract: Songbirds like the zebra finch (Taeniopygia guttata) communicate through learned vocalizations (songs). In the ascending auditory pathway, neurophysiological discrimination of learned vocalizations is most evident in portions of the caudomedial pallium (the "auditory lobule," AL). Initial exposures to a novel song trigger robust transcriptional changes in the AL, in particular causing a sharp increase in transcription of the EGR1 gene. However, this response habituates when the same song is repeated, suggesting acquisition of a learned memory. As EGR1 encodes a DNA-binding protein, we hypothesized that the EGR1 protein binds to gene targets that are involved in memory formation. Moreover, as EGR1 binding is sensitive to DNA methylation within its binding site, we hypothesized that changes in DNA methylation at EGR1 target sites contribute to the process of habituation. To test these hypotheses, Chromatin Immunoprecipitation for EGR1 was first combined with next-generation sequencing (ChIP-seq) to compare the ALs of adult male zebra finches in two conditions: 1) isolation overnight in silence; 2) hearing a novel song recording. The resulting data identified binding sites of EGR1 throughout the genome and provided evidence for shifts in binding at the time when novel song memories are being formed. In ongoing work, Reduced Representation Bisulfite Sequencing (RRBS) is being performed to identify changes in DNA methylation that may be linked to habituation. This is the first time EGR1 binding has been mapped in zebra finch and the first time DNA methylation has been investigated during song learning in the zebra finch.

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Poster

443. Genetic Approaches in Songbirds

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Topic: H.01. Animal Cognition and Behavior

Support: PRESTO program

JSPS KAKENHI 25115717

JSPS KAKENHI 16H03298

Title: CREB-mediated interplay of genes and environment in the postnatal song learning in songbirds

Authors: *K. ABE, D. WATANABE;
Kyoto Univ., Kyoto, Japan

Abstract: The development of behavioural traits in animals is influenced both by intrinsic and extrinsic factors. Vocal communication in humans and songbirds is one of the most prominent of such behaviours. The development of this ability, especially, requires social learning, that is, learning influenced by social interaction, education, or imitation, which enables the cultural transmission of information between generations. Previously, we have generated transgenic songbirds expressing enhanced or suppressed activity of CREB transcription factor. This parctical method to manipulate the genome of songbirds enables us to explore the molecular interdependence between intrinsic and extrinsic factors that influence song acquisition. Here we investigated the relationship between different learning situations and gene expression patterns during the postnatal development of vocal skills in zebra finches (*Taeniopygia guttata*). We found that inadequate song "tutoring" during development reduces the phosphorylation of CREB (c-AMP responsive element binding protein), alters the expression of genes containing CREB-binding elements, and reduces the quality of acquired songs. Transgenic manipulation to suppress CREB activity in zebra finches reduced song quality and auditory memory formation without affecting basal hearing ability or the acoustic quality of calls. Furthermore, although transgenic finches expressing constitutively active CREB were just as apt as wildtype finches to learn songs from a live tutor, in the absence of a live tutor these transgenic finches showed comparatively enhanced song acquisition, suggesting that the augmented CREB activity compensated for the lack of social input. Thus, different forms of transgenic CREB affected song acquisition, depending on the social condition. Our results provide an insight into the molecular mechanisms that determine how social influences affect the postnatal development of learned behaviours.

Disclosures: K. Abe: None. **D. Watanabe:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: Howard Hughes Medical Institute

Title: A role for SLIT1 in regulating singing behavior in adult male zebra finches.

Authors: *M. CHAKRABORTY[1], I. H. LEE[2], E. KOUNTOURIS[2], L.-F. CHEN[2], R. NEVE[3], J. CHABOUT[1], G. L. GEDMAN[2], H. CHOE[2], E. D. JARVIS[1];
[1]Neurobio., Howard Hughes Med. Inst., Durham, NC; [2]Neurobio., Duke Univ. Med. Ctr., Durham, NC; [3]McGovern Inst. For Brain Research, Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Vocal learning is a rare trait found in at least five mammalian and three independently evolved avian lineages. This trait is crucial for speech in humans, and can be attributed to specialized sensorimotor vocal circuits in the forebrain that possess unique connections compared to their adjacent brain areas. One hypothesis for evolution of vocal learning in vocal learning species are genetic differences that control and maintain the connections of forebrain vocal-motor pathways to brainstem vocal motor neurons. Transcriptome-scale gene expression studies indicate that the human laryngeal motor cortex (LMC), which controls the motor aspects of speech and song, is similar to the robust nucleus of the arcopallium (RA), the song production nucleus of songbirds. More than 55 genes contribute to their shared specialization, including genes that are enriched functionally for axon guidance, with downregulation of *SLIT1* being one of the most specialized. The *SLIT* family of genes acts through receptors of the *ROBO* family and controls commissural axon guidance, cell proliferation, and dendritic branching, among many other similar functions. To test the hypothesis that *SLIT1* gene expression specialization could be responsible for contributing to the specialized connection and thus production of learned vocalizations, we used an adeno-associated virus (AAV1) driven by a Super Core Promoter (SCP) to overexpress the human *SLIT1* gene bilaterally in RA of adult male zebra finches. We found that overexpression of human *SLIT1* caused birds (n = 14) to produce songs with reduced matches to their original song. These changes in song motif were characterized by decreases in overall song similarity of motifs, song accuracy, and song sequential match that reflect changes in temporal structure of songs, due to the birds having reduced stereotyped motif structure. In addition, we observed changes in song acoustic features including higher entropy and goodness of pitch. We did not observe these effects in control (n = 6) birds that received injections of an AAV virus expressing the reporter gene, green fluorescent protein (GFP) in RA. Ongoing experiments are examining the connectivity of the RA song nucleus in the *SLIT1* manipulated birds. Our results suggest that maintained differential downregulation of *SLIT1* in RA of adult birds is at least important to maintaining production of normal songs.

Disclosures: M. Chakraborty: None. **I.H. Lee:** None. **E. Kountouris:** None. **L. Chen:** None. **R. Neve:** None. **J. Chabout:** None. **G.L. Gedman:** None. **H. Choe:** None. **E.D. Jarvis:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: HHMI

Title: A comprehensive profiling of differential gene expression specializations in the song system of a vocal learning songbird, Taeniopygia guttata.

Authors: *G. GEDMAN[1], A. PFENNING[2], J.-N. AUDET[3], J. PALPENT[1], E. D. JARVIS[1,4]; [1]Duke Univ., Durham, NC; [2]Computat. Biol., Carnegie Mellon Univ., Pittsburgh, PA; [3]Biol., McGill Univ., Montreal, QC, Canada; [4]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Vocal learning is a rare and complex behavior seen in three to four independent lineages of birds and mammals, including in humans, which we use for speech and song. Among these avian lineages, the songbird has been the best studied. Their vocal learning circuit consists of an anterior forebrain pathway (AFP) containing pallial LMAN and striatal Area X nuclei involved in song learning, and the posterior forebrain pathway (PFP) containing pallial nuclei HVC and RA involved in song production. Analogous brain regions have been proposed for human speech, and recent transcriptomic analysis using microarrays suggests further convergence at the level of gene expression for the RA and Area X song nuclei to the human laryngeal motor cortex (LMC) and anterior striatal speech area respectively. HVC and LMAN did not exhibit significant convergence in gene specializations to speech regions in the human brain. These lack of molecular parallels may be attributed to not having profiled the surrounding cell populations, limited genes on microarrays, the computational tools used, or that parallels don't exist. Here, in an attempt to gain a more comprehensive view of the gene expression specializations necessary for vocal learning brain regions, we conducted RNA-seq transcriptome experiments of all four major song nuclei and the surrounding cell populations of the zebra finch. Brain regions were microdissected and RNA was sequenced using Illumina paired-end sequencing. We found that all nuclei exhibited more genes downregulated relative to their surround, suggesting that song nuclei develop with fewer of their respective pallial/striatal genes. Only 20 genes were similarly specialized in all song system nuclei, involved in a range of biological processes, including axon guidance, calcium signaling, and angiogenesis. LMAN had the lowest amount (233) of specialized genes, enriched for long-range projection development. HVC had the most (1172) specialized genes, enriched in cell signaling and nervous system development. Area X also had a large set of genes (946) specialized, enriched in neurogenesis and developmental functions. RA had an intermediate number (513) of specialized genes, enriched in angiogenesis and projection formation. Of the 55 genes found to be convergent

between songbird RA and human LMC in microarray experiments, 21 emerged in our RNA-seq dataset, including the axon guidance gene SLIT1. We plan to use these data in future comparisons to human cell type specific expression data to elucidate vocal learning analogs between humans and songbirds.

Disclosures: G. Gedman: None. **A. Pfenning:** None. **J. Audet:** None. **J. Palpent:** None. **E.D. Jarvis:** None.

Poster

443. Genetic Approaches in Songbirds

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Title: Transcriptome profiling of the vocal nuclei in zebra finches (T. guttata) reveals molecular specializations of neural circuits for the production of learned song

Authors: *S. FRIEDRICH, P. V. LOVELL, C. V. MELLO;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Vocal learning is a behavioral trait that enables animals to acquire vocalizations through imitation. This rare trait is prevalent in songbirds and is subserved by a set of interconnected brain nuclei that control and coordinate vocal and respiratory organs. The connectivity and physiology of these vocal control nuclei have been extensively studied in songbirds, and in particular in zebra finches (*T. guttata*). In contrast, even though there has been some progress in examining gene expression profiles of vocal nuclei, our understanding of their molecular organization and specializations is still limited. Previous efforts using cDNA (Lovell et al., 2008) and short oligo microarrays (Hilliard et al., 2012; Hara et al., 2012; Whitney et al., 2015; Pfenning et al., 2015) have identified differentially expressed transcripts in several zebra finch vocal nuclei, including HVC, RA, and nXIIts of the direct vocal motor pathway, and striatal area X within the anterior pathway for vocal plasticity. However, these reports were mainly focused on identifying differentially expressed transcripts that are convergently shared with other avian vocal learning groups (i.e. parrots and hummingbirds) and with humans. Here we report a detailed and extensive transcriptome profiling analysis of these vocal nuclei in zebra

finches, based on a reanalysis of publically available microarray data from the Mello, Jarvis, and White labs. Our efforts include a comprehensive genome-based curation and annotation of oligo arrays from the Jarvis lab, the identification of possible transcript variants that may explain some of the expression profiling variability, and the definition of reliable significance cut-off based on the use of confirmatory in situ hybridization data derived from the ZEBrA online database (www.zebrafinchatlas.org). Bioinformatics analyses reveal evidence for the differential regulation of numerous genes that are involved in neuronal excitability and plasticity (e.g. ion channels, transmitters, neuromodulators, and their receptors), as well as circuit connectivity (e.g. axon guidance cues). These findings provide novel insights into molecular specializations of vocal control circuits in finches, identify candidate pathways related to patterns of connections between vocal nuclei, and point to candidate target genes for further mechanistic and regulatory studies.

Disclosures: **S. Friedrich:** None. **P.V. Lovell:** None. **C.V. Mello:** None.

Poster

443. Genetic Approaches in Songbirds

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 443.08/AAA22

Topic: F.01. Neuroethology

Support: NSF Grant 1456302

Title: Comparative transcriptomics of vocal nuclei of Anna's hummingbirds and zebra finches reveal convergent and group-specific molecular features of vocal learning circuits in hummingbirds and songbirds

Authors: *P. V. LOVELL, M. WIRTHLIN, C. V. MELLO;
Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: Vocal learning is a rare behavioral trait that enables animals to acquire their vocal repertoires though imitation, providing a substrate for speech and language development in humans. Among birds, recent phylogenomics studies have helped establish that vocal learning evolved independently at least twice, namely in hummingbirds and in songbirds/parrots (the latter now being considered sister taxa with the trait likely originating in a common ancestor). Identifying features of the vocal learning circuitry that may have evolved convergently across these avian vocal learner groups offers unique opportunities for identifying fundamental requirements and principles associated with this trait. With this goal in mind, we have analyzed the transcriptomes of key vocal nuclei that are present in the Anna's hummingbird, a known

vocal learner, in comparison with analogous nuclei in zebra finches, a songbird species and a model organism for vocal learning research. We focused on identifying transcripts that are differentially expressed in the three primary nuclei of the direct vocal control pathway, namely HVC, RA, and nXIIts (data for the latter two nuclei were made publically available by the Jarvis lab). Because samples from the Anna's hummingbird were cross-hybridized to oligo-based (Pfenning et al., 2014) and cDNA-based microarrays (Lovell et al. 2008) that were specifically developed for zebra finches, several preparatory steps had to be applied, including a careful curation of oligo and EST sequences in the context of cross-alignments to the Anna's hummingbird genome, and an analysis of splice variants contributing to array signal variability. Our analysis has revealed several hundred transcripts that are significantly up- or down-regulated in all three nuclei compared to adjacent brain tissues. Intriguingly, while a subset of these transcripts appear to be unique to the Anna's vocal control system, and thus may reflect properties unique to vocal learner hummingbirds, other subsets were shared with zebra finches and may reflect more broadly required features of vocal learning systems. Of interest, both shared and unique subsets include genes involved in several aspects of neuronal structure and physiology, including ion channels, neurotransmitter receptors, modulators of synaptic structure and function, and modulators of long-distance connectivity. Together, these findings shed light on possible constraints on the evolution of avian vocal learning circuits that may reflect fundamental requirements of vocal learning systems. They also identify genes that represent choice candidate targets for mechanistic studies involving gene manipulation.

Disclosures: P.V. Lovell: None. **M. Wirthlin:** None. **C.V. Mello:** None.

Poster

443. Genetic Approaches in Songbirds

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Program#/Poster#: 443.09/AAA23

Topic: F.01. Neuroethology

Support: NIH DC014432

NIH GM092842

Title: Identification of candidate gene regulatory sequence elements associated with the emergence of vocal learning in songbirds

Authors: *M. WIRTHLIN, P. V. LOVELL, C. V. MELLO;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: How does a novel behavior evolve at the level of the genome? The occurrence of novel genes, as well as mutations in protein-coding sequences that alter gene function, have been demonstrated in the genomes of vocal learning songbirds compared to vocal non-learner outgroups (Wirthlin et al, 2014). However, such events are rare, and do not sufficiently explain the emergence of specialized brain nuclei for the acquisition and production of learned vocal behavior. In contrast, the anatomical, molecular, and physiological properties of these specialized nuclei have been linked to drastic changes in the expression of pre-existing genes. These changes in gene expression are thought to be the result of changes in cis-regulatory elements associated with these genes. However, identifying the critical regulatory sequences underlying the expression of genes in specific brain regions remains a fundamental challenge in neurogenomics. In order to identify the changes in regulatory sequence that may be associated with the emergence of vocal learning circuits, we developed a pipeline for comparative brain-expressed gene promoter analysis. We used multiple expressed sequence databases to annotate transcriptional start sites in the reference genome of a model vocal learning species, the zebra finch. We next identified transcription factor binding site motifs that are enriched in the promoters of genes selectively expressed in vocal control nuclei, which are present only in vocal learning species - these were considered candidate vocal learning regulatory motifs. We then identified orthologous promoters in additional high quality avian genomes, and determined which candidate motifs were enriched only in orthologous promoters in songbirds, versus those that had preadapted in non-learner lineages. We discuss the most promising regulatory motifs identified and their putative target gene networks in the context of their known biological functions, and highlight examples of genes that could subserve the known molecular and physiological properties of brain nuclei critical for learned vocal behavior. These analyses provide a framework for understanding how shifts in gene regulatory sequence composition could lead to the emergence of vocal learning circuits. They also provide a general roadmap for integrating knowledge from genomics, gene expression analysis, neuroanatomy, physiology, and phylogenetics to provide novel insights into the evolution of behavior.

Disclosures: **M. Wirthlin:** None. **P.V. Lovell:** None. **C.V. Mello:** None.

Poster

443. Genetic Approaches in Songbirds

Location: Halls B-H

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Program#/Poster#: 443.10/AAA24

Topic: F.01. Neuroethology

Support: NIH Grant DC014432

NIH Grant GM092842

Title: An update and new applications of the zebra finch expression brain atlas (www.zebrafinchatlas.org)

Authors: *C. V. MELLO, P. V. LOVELL;
Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: The Zebra finch Expression Brain Atlas (ZEBrA; www.zebrafinchatlas.org) is a publicly accessible online resource for investigating the brain distribution of genes involved in the physiology, development, and maintenance of functional circuits in the brain of songbirds. It consists of a comprehensive in situ hybridization database representing the expression patterns for a large set of transcripts in brain of adult male zebra finches (*T. guttata*), a representative songbird species. Its major features include: (1) The In situ database - a collection of high-resolution (0.46 μm/pixel) digital images presented along with annotated drawings from a reference Histological Atlas. ZEBrA currently houses more than 2,000 images (>100 GB) corresponding to ~500 brain expressed genes, including markers of all major nuclei that comprise the song system. (2) A reference Histological Atlas Browser - A set of 18 annotated drawings prepared in registration with Nissl- and Myelin-stained images of sagittal brain sections derived from the Karten/Mitra atlas. (3) A Gene Family Search Tool - A feature that facilitates searches for genes based on their membership in specific gene families. (4) A Neuroanatomical Marker Search Tool - A search engine that allows users to retrieve a list of genes that are markers of a given structure, or of multiple structures. Recent applications of ZEBrA include: (1) utilizing the comprehensive in situ database to validate and establish cut-off criteria for transcriptomics experiments utilizing laser capture microdissections and microarrays; (2) performing quantitative analysis of regional gene expression patterns to identify molecular signatures of specific brain areas in zebra finches; (3) obtaining evidence of heterogeneity of cellular patterns of gene expression as a basis for cellular phenotyping within vocal control nuclei; (4) utilizing the ZEBrA platform and its embedded features as a resource that can be adopted by individual labs for cataloguing, storing, and accessing high resolution brain gene expression data.

Disclosures: C.V. Mello: None. **P.V. Lovell:** None.

Poster

443. Genetic Approaches in Songbirds

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Program#/Poster#: 443.11/AAA25

Topic: F.01. Neuroethology

Support: Sally McDonnell-Barksdale Honors College

Title: Egg laying male has androgynous song system

Authors: *J. A. HOWELL, N. WEBB, R. PEREZ, A. HRIBAR, L. DAY;
Univ. of Mississippi, University, MS

Abstract: Zebra finch sexes differ in plumage, song nuclei, and behavior. The effects of sex chromosome genes, autosomal genes, and hormones to sexual differentiation are not completely understood. Unusual birds that have both male and female traits help us understand sexual differentiation. A "chimera" in our aviary had male plumage and a male partner, laid eggs, and produced viable offspring. Using mate preference tests we found that the chimera and its lineage were less preferred as mates than controls, suggesting differences making them unattractive to other birds in the aviary. Males of the chimeric lineage also had greater same-sex mate preferences than control males; chimeric lineage and control females did not differ. Sampling blood, eggs, gonads, sexually dimorphic feathers and skin, beaks, and hearts, all chimeric tissues have ZW female chromosomes. Thus, only the plumage of the chimera suggested any abnormalities. To compare the song system of the chimera to males and females, brain tissue of the chimera, male partner, and a female control was cut at 30 μm, and every third slice was mounted for Nissl staining. We measured volume, cell number, and cell size of three sexually dimorphic song nuclei. The proportions for male to female were consistent with other studies. Our chimera is between the two. For instance, male:female ratio for RA is ~5.74. Our male:female RA ratio is 4.81 and male:chimera RA ratio is 1.30. For LMAN, the male:female ratio is 5.10 and male:chimera ratio is 1.83. HVC could not be seen in the female due to errors in coverslipping, but the male:female HVC ratio is ~6.20. Our male:chimera HVC ratio is 1.74. Area X is prominent in male zebra finches but does not appear in females. However, we have identified a putative X in our chimera. We will use other X markers to verify this. In HVC, RA, and LMAN, the cell number and relative volume tended to be doubled in the male compared to the chimera, but quadrupled in the male compared to the female. Cell soma size was similar for the male and chimera for HVC. For LMAN, soma size ratios were 1.73 for male:chimera and 1.43 for male:female. Other males and females will be measured to determine where the chimera is in the standard male/female range for these measures. These results suggest that although the chimera had a full female genotype, it had a partially masculinized song system. We will be analyzing video recordings of the chimera and its mate to find out if the chimera produced song and to observe nesting and mating behaviors. Other studies with a similar chimeric bird, with male plumage and a female reproductive system, had a fully female song system suggesting multiple pathways for some shared phenotypic traits.

Disclosures: **J.A. Howell:** None. **N. Webb:** None. **R. Perez:** None. **A. Hribar:** None. **L. Day:** None.

Poster

443. Genetic Approaches in Songbirds

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Program#/Poster#: 443.12/AAA26

Topic: F.01. Neuroethology

Title: Expression of lipoprotein receptor-related proteins (LRPs) in brain of zebra finches: possible links to VSVg-pseudotyped viral infectivity

Authors: *T. VELHO[1,2], P. V. LOVELL[3], M. WIRTHLIN[3], C. LOIS[1], C. V. MELLO[3]; [1]Biol. and Bioengineering Dept., CALTECH, Pasadena, CA; [2]Brain Inst., Federal Univ. of Rio Grande do Norte, Natal, Brazil; [3]Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Low-density lipoprotein receptor (LDLR) is thought to be the primary receptor through which viral particles pseudotype with the vesicular stomatitis virus glycoprotein (VSVg) gain entry into cells. The lipoprotein receptor-related proteins (LRPs) appear to function as alternate, secondary receptors for the same VSVg. Intriguingly, VSVg-pseudotyped lentiviral infectivity in songbirds, such as the zebra finch, appears to be much less efficient when compared to mammals, as reflected in a smaller percent of infected cells upon lentiviral injections into different tissues, including the brain. To investigate whether the empirical species differences in infectivity levels are due to biological differences in receptor expression, we set out to examine the zebra finch orthologs of LDLR and LRPs to determine their expression levels in the brain. Intriguingly, comparative genomics analysis indicates that the zebra finch LDLR is highly divergent and truncated compared to orthologs in both chicken and mammals, and is possibly pseudogenized. We have also identified at least 10 distinct LRP genes in the zebra finch genome, which in constrast with LDLR show a high degree of sequence conservation when compared to mammalian orthologs. Of these, we have successfully examined the brain expression of 8 genes by in situ hybridization, and found convincing evidence for brain expression for 6 finch LRPs, with varying levels or degrees of cellular and regional specificity. In particular, LRP1 nad LRP8 show robust brain expression, and the relative distribution of labels cells suggest the possibility that their expression may be restricted to specific cell types. Studies are currently underway to assess whether distinct cell types in the songbird brain (e.g. GABAergic vs Glutamatergic) express unique combinations of LRPs, and to further address the possibilitiy that one or more LRP may be critical for mediating VSVg-dependent viral infectivity in finch brain tissue.

Disclosures: **T. Velho:** None. **P.V. Lovell:** None. **M. Wirthlin:** None. **C. Lois:** None. **C.V. Mello:** None.

Poster

443. Genetic Approaches in Songbirds

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Program#/Poster#: 443.13/BBB1

Topic: F.01. Neuroethology

Support: Canadian Institute for Advanced Research (CIFAR)

Title: Robust male-specific expression of the UTS2B gene emerges early in the development of a zebra finch forebrain vocal control nucleus

Authors: *Z. W. BELL, J. M. GEORGE, D. F. CLAYTON;
Sch. of Biol. and Chem. Sci., Queen Mary, Univ. of London, London, United Kingdom

Abstract: The UTS2B gene encodes an octapeptide, which is highly expressed in the spinal cord and brainstem of mammals, with functional studies focusing mainly on vasoregulation. Unexpectedly, several transcriptomic analyses have suggested that UTS2B is expressed in or around parts of the songbird brain involved in vocal communication and perception. The objective here was to use *in situ* hybridization to localize the expression of UTS2B in the zebra finch brain. As in mammals, the mRNA was detected in brainstem nuclei. However, it was also detected in cells scattered throughout the forebrain, with a striking concentration of cells specifically within the song control nucleus HVC. Concentrated expression in HVC was observed only in males, and was already evident at 15 days post-hatch, well before the functional maturation and sexual differentiation of the song control circuit. The expression showed no noticeable *diel* changes or activity dependence. HVC is a key part of the telencephalic song control circuit, which is unique to oscine songbirds. Thus evolution of the song control circuit may be linked to genomic changes that expanded the expression of UTS2B into the forebrain, especially into HVC. While the functional significance of this remains unknown, it suggests a potentially broader role for UTS2B in the evolution and control of complex behaviours.

Disclosures: **Z.W. Bell:** None. **J.M. George:** None. **D.F. Clayton:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: NIHT32HD07228

NIHR01MH070712

NSFIOB0924143

Title: Reelin signaling in the basal ganglia: striatal, pallidal, or both?

Authors: *E. FRALEY[1], P. E. PHELPS[2], S. A. WHITE[2];
[1]UCLA, Santa Monica, CA; [2]UCLA, Los Angeles, CA

Abstract: The aim of this investigation is to determine how Reelin signaling influences basal ganglia function, with a focus on vocal communication. Like human speech, birdsong is a learned vocal behavior. We previously established that, like the human speech-related transcription factor FoxP2, members of the Reelin-signaling pathway are also regulated by singing behavior. This behavioral driven regulation is particular to the striato-pallidal song nucleus, area X. Reelin signals through high affinity receptors appolipoprotein receptor 2 (Apoer2) and very-low density lipoprotein receptor (Vldlr) to initiate phosphorylation of the intracellular signaling molecule Disabled-1 (Dab1). Reelin signaling has established roles in regulating neuronal migration and modulating dendritic morphology and plasticity. To dissect which cells express what components of the Reelin-signaling pathway, we drew from our cross-species investigation of the murine basal ganglia. In mice, we identified cells in the globus pallidus that express Dab1 and cells in the anterior striatum that express Reelin. We then hypothesized that pallidal cells in zebra finch area X would also be Dab1 positive. Indeed, using co-immunostaining and tracing techniques we have now identified Dab1 positive cells in area X that are pallidal. Additionally, the wide expression of Vldlr includes both striatal and pallidal cells in area X. We thus conclude that Reelin signaling is likely to not only influence cortico-striatal synapses, but also the striato-pallidal synapses; thereby impacting output of area X to the downstream thalamic nucleus in the zebra finch song control circuit. Activity-dependent enhancement of dendritic morphology of subsets of cells in both striatum and pallidum may thus be mediated by Reelin signaling. This phenomenon may not be exclusive to the zebra finch, and we suggest a striato-pallidal Reelin signaling mechanism exists in the mouse; although vocal regulation of gene expression changes in this species has yet to be determined.

Disclosures: **E. Fraley:** None. **P.E. Phelps:** None. **S.A. White:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: NIH Grant MH070712-09

NIH Grant NICHD T32HD07228

Title: FoxP2 overexpression coupled with auditory deprivation in adult zebra finches disrupts molecular microcircuitry in a song-dedicated basal ganglia nucleus

Authors: *N. F. DAY[1], Z. D. BURKETT[1], A. T. HILLIARD[2], X. XIAO[1], S. A. WHITE[1]; [1]Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA; [2]Dept. of Biol., Stanford Univ., Palo Alto, CA

Abstract: Human mutations in the FOXP2 transcription factor result in language disorders and altered basal ganglia structure. The neural underpinnings of speech learning can be investigated in songbirds because, like speech, birdsong is learned through social interactions, relies on auditory feedback and cortico-basal ganglia circuitry, and compensates for experimentally-induced errors. In the songbird brain, most FoxP2-enriched areas (e.g. cortex, thalamus) show a static expression level, whereas Area X (a song-dedicated basal ganglia nucleus), shows dynamic regulation: *FoxP2* mRNA and protein decrease when juvenile and adult males sing, after which songs are more variable. This 'on-line' regulation during singing is critical for song learning, but the role of FoxP2 in the active maintenance of a learned vocal motor skill is poorly understood. What is known includes that auditory feedback is crucial for the learning and the maintenance of song and speech, because deafness in adulthood causes both to deteriorate. In songbirds, hearing and FoxP2 are linked: The more a bird hears itself practice, the lower its Area X FoxP2 levels; no such correlation is observed in deaf birds.

To investigate the post-developmental role of FoxP2 in adult zebra finches, we used an adeno-associated virus (AAV) to constitutively augment levels of FoxP2 in Area X to test whether song maintenance and gene networks in the mature organism are altered. Birds were injected with either GFP- or FoxP2-overexpressing AAV constructs; a subset of each group were then deafened. We examined the syntactical and phonological changes that accompany auditory deprivation following FoxP2 (or GFP) overexpression. We then performed RNAseq on tissue punches of Area X from these same birds and conducted Weighted Gene Co-Expression Analysis (WGCNA). Gene networks were correlated to song behaviors to determine suites of genes that are regulated by singing, auditory state, or overexpression of two FoxP2 isoforms. These analyses provide clarification of FoxP2 targets within the basal ganglia. They also reveal changes to the 'molecular microcircuitry' of Area X that occur when behavior-driven cycles of

FoxP2 are interrupted by overexpression and/or after prolonged auditory deprivation. Elucidation of these molecular events will launch novel investigations into genes or regulatory pathways that are critical for the ongoing maintenance of a procedurally-learned task that underlies human speech and language.

Disclosures: N.F. Day: None. **Z.D. Burkett:** None. **A.T. Hilliard:** None. **X. Xiao:** None. **S.A. White:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: NIH Grant MH070712-09

NIH Grant NICHD T32HD07228

Title: FoxP2 isoform-specific overexpression in juvenile zebra finches alters transcriptional networks underlying learned vocalization.

Authors: *Z. D. BURKETT[1,2], N. F. DAY[1], A. T. HILLIARD[4], J. B. HESTON[3,1], X. XIAO[1,2], S. A. WHITE[1,2,3];
[1]Integrative Biol. & Physiol., [2]Interdepartmental PhD Program in Molecular, Cellular, and Integrative Physiol., [3]Neurosci. Interdepartmental PhD Program, Univ. of California Los Angeles, Los Angeles, CA; [4]Dept. of Biol., Stanford Univ., Stanford, CA

Abstract: The transcription factor FoxP2 is necessary for proper development of learned vocalizations in humans and songbirds. In the zebra finch, FoxP2 is dynamically downregulated in the basal ganglia song control nucleus Area X during vocal practice in juvenile and adult birds. When this behavioral downregulation is blocked during vocal learning by knockdown or overexpression of FoxP2, adult vocalizations are similarly poor. This suggests that behavior-linked cycling of the molecule is critical for learning. In addition to the 'full-length' isoform, a truncated version of FoxP2 that is capable of dimerizing but incapable of binding to DNA exists in both humans and zebra finches. This isoform perhaps functions as a post-translational regulator of other FoxP2 isoforms, though its role in vocal learning has been uninvestigated. Using viral vectors, we overexpressed the full-length or truncated isoforms of FoxP2 in Area X during vocal learning. We observed alterations to the birds' vocal phenotypes specific to the overexpressed isoform. The data replicate our prior findings for the full length FoxP2, provide

new insight regarding the truncated isoform, and indicate that the dynamic regulation of each isoform contributes uniquely to vocal learning and variability. We then performed RNA-sequencing and weighted gene coexpression network analysis on Area X micropunches from these animals to understand how dysregulation of these isoforms and their concurrent changes in vocal behavior are reflected in the transcriptome.

Disclosures: Z.D. Burkett: None. **N.F. Day:** None. **A.T. Hilliard:** None. **J.B. Heston:** None. **X. Xiao:** None. **S.A. White:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: NIH R21HD065271

DOD AR093327

Title: Attenuated expression of contactin associated protein-like 2 in a primary motor nucleus of the song system impairs vocal imitation

Authors: *S. A. WHITE[1], Q. CHEN[2], Y. MAI[2], M. C. CONDRO[2], S. C. PANAITOF[3]; [1]Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA; [2]Univ. of California, Los Angeles, CA; [3]Univ. of Nebraska, Kearney, CA

Abstract: Human mutations in contactin associated protein-like 2 (CNTNAP2) are associated with cortical dysplasia-focal epilepsy, autism spectrum disorder, and specific language impairment. Across these diffusely debilitating disorders, deficits in speech and language are a common theme. Songbirds are useful models for the study of human speech disorders due to the significant similarities between the learning of song and speech. The laryngeal motor cortex, a language control region in the human brain shares striking genetic and anatomical similarities with the robust nucleus of the arcopallium (RA), the primary vocal control nucleus in songbird cortex. These include shared gene expression profiles and direct cortical projections onto the motor neurons that control the muscles of phonation. In the zebra finch (*Taeniopygia guttata*), song learning and the underlying neural circuitry are sexually dimorphic; males, but not females, learn their courtship songs using an interconnected set of song-dedicated brain regions. Within RA, Cntnap2 expression becomes sexually dimorphic during the sensorimotor phase of song learning: Males retain high levels whereas expression declines in females. Interestingly, the

divergence in expression appears due to the shorter isoform of this neurexin-like protein. To begin to assess the role of Cntnap2 specifically in vocal learning, here, we developed RNAi constructs to attenuate levels of both long and short zebra finch Cntnap2 isoforms. After confirming the knockdown effect in a human cell line and in primary cultures of zebra finch telencephalon, we stereotaxically injected an adeno-associated virus (AAV) bearing the RNAi constructs in vivo into RA at the onset of sensorimotor learning. Pupils who received the targeting RNAi failed to accurately imitate their tutor's song, omitting a higher percentage of syllables compared with siblings who received a non-targeting construct. The targeting RNAi did not interfere with the pupil's ability to modify its own song over the course of sensorimotor learning. Sholl analysis revealed that attenuation of Cntnap2 altered dendritic morphology of RA neurons, providing a possible mechanism underlying the vocal deficits. These results suggest that among Cntnap2's many functions within the nervous system, its expression within the cortical vocal control region alone is critical for accurate vocal imitation.

Disclosures: S.A. White: None. **Q. Chen:** None. **Y. Mai:** None. **M.C. Condro:** None. **S.C. Panaitof:** None.

Poster

443. Genetic Approaches in Songbirds

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Topic: F.01. Neuroethology

Support: JSPS KAKENHI Grant Number 26001737

Title: Accumulation of singing experience regulates the critical period of vocal plasticity during birdsong active learning

Authors: *S. HAYASE, M. KOBAYASHI, E. OHGUSHI, K. WADA;
Hokkaido Univ., Sapporo/Hokkaido, Japan

Abstract: Motor learning processes, such as human language acquisition or birdsong learning, require repeated and voluntary practice during a critical period in ontogeny. During these motor learning processes, it remains unclear how the critical period is regulated. Past studies shed light on the effect of "passive" experience, *i.e.* reception of sensory stimuli, on the regulation of critical periods in sensory systems. To reveal the contribution of "active" motor experience for the critical period of motor learning, we focused on songbirds, which possess an analogous vocal system to human and learn a song in juvenile stage. In the song system of songbirds, a number of neuroplasticity-related genes are induced by singing behavior. *Arc*, a neuroplasticity-related IEG,

was strongly induced by singing in juveniles than adults in pallial nuclei RA in the song system; an analogous region to motor cortex layer V in mammals. This suggests a potential effect of singing experience-dependent and brain region-specific motor-driven genes on the regulation of the critical period of vocal learning. Then, we inhibited the singing behavior of juveniles until maturity to suppress singing-driven IEGs induction and examine the effects on vocal development. The singing-inhibited birds produced highly plastic vocal patterns at adult stage, while the dendritic spine density in RA projection neurons showed juvenile-like values, indicating that the accumulation of singing-experience, not aging, is crucial for regulation of vocal plasticity. To elucidate the molecular mechanisms underlying such accumulation of singing-experience in the vocal system, we performed RNA-seq and co-expression analysis in RA on a genome-wide scale. As a result, a total of 126 motor-driven genes were regulated by the accumulation of singing-experience, including epigenetic regulators. These genes were strongly induced by singing in RA of juveniles and singing-inhibited birds but not in freely singing adults. In addition, the vocal learning ability of singing-inhibited birds was preserved. These results suggest that a set of motor-driven IEGs engages as a count accumulator of voluntary singing experience to coordinate the critical period of vocal learning.

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

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

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Program#/Poster#: 444.01/BBB7

Topic: F.01. Neuroethology

Title: Vocal combinatorial behavior in rats

Authors: ***T. RIEDE**, C. HERNANDEZ, M. SABIN, H. BORGARD;
Midwestern Univ., Glendale, AZ

Abstract: The coordination between breathing and laryngeal motor control is critical for normal vocal production, including ultrasonic vocal behavior in laboratory rats. Features of fundamental frequency of ultrasonic calls depend on specific activity patterns of intrinsic laryngeal muscles and muscles contributing to the build- up of subglottal pressure. Rats concatenate different call types of their 50-kHz repertoire into single utterances, i.e. calls are produce in short succession during the same breathing cycle. We now describe how rats also combine and recombine 22-kHz and 50-kHz calls into single composite calls. Twentytwo-kHz and 50-kHz ultrasonic calls are well-characterized different call types, yet they are sometimes concatenated into one breath and are part of the same call. The 22-50 composite calls were found in males during the initial

encounter with a female. The finding demonstrates the existence of a flexible laryngeal-respiratory coordination in rats. Combining two calls either into a single breath or into two subsequent breathing cycles has consequences for vocal activity and possibly the function of vocal signals.

Disclosures: **T. Riede:** None. **C. Hernandez:** None. **M. Sabin:** None. **H. Borgard:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.02/BBB8

Topic: F.01. Neuroethology

Title: Male mice emit ultrasonic vocalizations during agonistic interactions

Authors: D. T. SANGIAMO, M. R. WARREN, X. ZHONG, *J. P. NEUNUEBEL; Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: In the animal kingdom, innate social behaviors play a crucial role in survival and reproduction. For example, agonistic behaviors between two males such as fighting and chasing shape the formation of hierarchies, which allows the dominant male to access food, territory, and females (Dewsbury, 1982). In mice, agonistic behaviors are accompanied by ultrasonic vocalizations (USVs) (Gourbal et al., 2004) although some reports suggest that USVs are only produced during reproductive behaviors (Whitney et al., 1973). Conflicting evidence as well as limitations in the ability to determine which mice are vocalizing in a social context makes the relationship between aggressive behavior and USVs unclear. To address this problem, a microphone-array based system was used to triangulate the location of USVs from groups of freely interacting mice (6 groups of 2 males and 2 females; 12-21 weeks old B6.CAST-Cdh23Ahl+/Kjn) and assign the vocalizations to specific animals. A total of 108,213 vocalizations were detected with 37.4% assigned to specific mice. Males produced 81.6% of the assigned vocalizations and 18.4% were emitted by females. To examine the relationship between aggressive behavior and USVs, a machine based-learning program was used to determine when males were chasing and fighting each other (Kabra et al., 2012). There were 411 fights detected (mean duration = 3.44 seconds; SEM = 0.13). In every experiment, each male chased the other male (n = 723; mean duration = 1.06 seconds; SEM = 0.03). The male vocalization rate was examined before, during, and after fights and chases. For fights, the vocalization rates were significantly different between each of the three periods (mean vocalization rate before = 1.32 Hz; SEM = 0.11; during = 1.77; SEM = 0.10; after = 0.74; SEM = 0.09; 1-way ANOVA, $F_{2,1230}$= 27.0, $p < 10^{-11}$). Male mice vocalized at a higher rate during fights than before or after fighting

(Tukey-Kramer; before, $p < 0.005$; after, $p < 10^{-9}$). In addition, males vocalized at a higher rate before fights than after them (Tukey-Kramer; $p < 10^{-4}$). Male vocalizations were observed before, during, and after chases (mean vocalization rate before = 1.19 Hz; SEM = 0.08; during = 0.99; SEM = 0.07; after = 1.15; SEM = 0.08); however, there were no significant differences between the periods (1-way ANOVA; $F_{2,2166} = 2.0$; $p = 0.13$). These results show that male mice vocalize during their agonistic interactions in competitive living conditions. In the future, our work will focus on classifying more aggressive behaviors and looking at whether specific types of vocalizations co-occur with specific aggressive behaviors.

Disclosures: D.T. Sangiamo: None. **M.R. Warren:** None. **X. Zhong:** None. **J.P. Neunuebel:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.03/BBB9

Topic: F.01. Neuroethology

Title: Sex differences in the acoustic structure of mouse ultrasonic vocalizations

Authors: M. S. SPURRIER[1], *E. ROTH[2], M. R. WARREN[1], J. P. NEUNUEBEL[1];
[1]Dept Psychological and Brain Sci., [2]Dept Neurobiol, Univ. of Delaware, Newark, DE

Abstract: During social interactions, mice produce ultrasonic vocalizations (USVs) (Sales, 1972). These vocalizations are primarily attributed to males when they interact with a female (Whitney et al., 1973; Warburton et al., 1989), although evidence shows that vocalizations are emitted when two female mice interact (Maggio and Whitney, 1985; Moles et al., 2007). Recently, work from Neunuebel et al. (2015) indicated that male and female mice housed in large, mixed sex groups vocally interact during courtship; however, it is unknown if female mice vocalize in the presence of males during dyadic interactions. This gap in knowledge exists because prior research was unable to identify which mouse was vocalizing during social interactions. To overcome this challenge, we used an 8-channel microphone-array based system to record vocalizations and behavior from freely interacting dyads of male and female mice for 30 minutes (9-21 weeks old B6.CAST-Cdh23Ahl+/Kjn mice). Thirty minutes prior to the start of the recording session, non-invasive lavage and cytological assessment of vaginal cells were performed. Sexually experienced female mice in estrus were randomly paired with novel, yet sexually experienced male mice. A total of 41,436 vocalizations were detected (median = 5,390; IQR = 1,459-5,710) with 41.8% assigned to specific mice. For the assigned vocalizations, 85.1% were male and 14.8% were female. Although both sexes vocalized, male mice vocalized

significantly more than females (median male count = 1,966; IQR = 575-2,141; median female count = 294; IQR = 71-372; Mann-Whitney U-test, $z = 3.0$, $p < 0.005$). Next, we investigated potential sex differences in vocal production by calculating the duration, bandwidth, and pitch of the vocalizations. When comparing the duration of male and female vocalizations, no difference was detected (median male = 17.4 msec; IQR = 12.9-27.5; median female = 18.1; IQR = 13.2-27.3; Mann-Whitney U-test, $z = -1.6$, $p = 0.11$). In contrast, the bandwidth of vocalizations was significantly larger for males than females (median male = 10.1 kHz; IQR = 6.8-14.8; median female = 7.5; IQR = 5.7-10.9; Mann-Whitney U-test, $z = 20.0$, $p < 10^{-88}$). This resulted from male mice vocalizing at considerably higher frequencies than females (median male = 79.1 kHz; IQR = 71.6-83.8; median female = 75.6; IQR = 69.1-83.2; Mann-Whitney U-test, $z = 9.2$, $p < 10^{-19}$), since there was no difference between low frequencies (median male = 65.6 kHz; IQR = 60.9-72.6; median female = 65.8; IQR = 60.1-73.2; Mann-Whitney U-test, $z = -1.5$, $p = 0.14$). These results reveal sex differences in the acoustic structure of vocalizations recorded when pairs of male and female mice socially interacted.

Disclosures: **M.S. Spurrier:** None. **E. Roth:** None. **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.04/BBB10

Topic: F.01. Neuroethology

Title: Direct quantification of a social communication deficit in a mouse model of autism

Authors: ***M. R. WARREN**, J. P. NEUNUEBEL;
Univ. of Delaware, Newark, DE

Abstract: Autism is a behaviorally defined disorder characterized by social impairments and vocal deficits. A number of mouse models of autism exist that exhibit these symptoms (Bader et al., 2011; Tania et al., 2013), but a comprehensive understanding of the impairments has been hampered by the inability to localize vocalizations to a particular mouse as animals freely interact in complex, dynamic social contexts. To address this, a sound source localization system was used to simultaneously record video and audio from groups (2 males and 2 females, 8-12 weeks of age) of mouse models of autism (Cacna1c<tm2ltl>/J, n=6), as well as their wild-type littermates (n=5) over a period of five hours. Social interactions were quantified by calculating the total duration each animal spent near another animal over 20 minute intervals. We showed that male Cacna1c mice spent significantly less time interacting with their male counterparts as

compared to their wild-type littermates (2-way ANOVA, $F_{1,14} = 3.2$, $p<10^{-3}$). Moreover, for both groups the interactions significantly decreased over time ($F_{1,14} = 25.2$, $p < 10^{-3}$). When two females interacted, no differences in time (2-way ANOVA, $F_{1,14} = 0.7$, $p = 0.74$) or genotype ($F_{1,14} = 3.0$, $p = 0.09$) were observed between the Cacna1c and wild-type mice. To assess the interplay between social behavior and vocalizations, we analyzed the vocal activity of two mice while they were socially interacting. We discovered that the vocal activity was considerably lower in the Cacna1c mice than in wild-type animals. For both the Cacna1c and wild-type animals, the vocal activity decreased over time (2-way ANOVA, $F_{1,14} = 5.2$, $p < 0.03$; $F_{1,14} = 7.6$, $p < 10^{-10}$). The vocal activity of the females during their interactions was significantly lower in the Cacna1c mice compared to wild-type mice (2-way ANOVA, $F_{1,14} = 9.3$, $p < 0.003$); however, there was no difference over time ($F_{1,14} = 0.6$, $p = 0.88$). To investigate potential deficits in vocal exchanges for both males and females, we compared the number of social interactions where both animals vocalized. There were notable differences between Cacna1c and wild-type mice (males; $\chi^2 = 83.5$; $p < 10^{-10}$; females; $\chi^2 = 29.6$; $p < 10^{-7}$). For the male mice, wild-type animals jointly vocalized in 23.9% of the social interactions compared to 16.2% for Cacna1c mice. Cacna1c and wild-type females jointly vocalized in 2.8% and 5.4% of their interactions, respectively. By tracking the vocal behavior of individual mice as they interacted, we revealed a striking communication deficit in a mouse model of autism.

Disclosures: **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.05/BBB11

Topic: G.02. Motivation

Support: NSF Grant 0953106

Title: Dopaminergic mechanisms of vocal communication in prairie voles

Authors: ***M. L. GUSTISON**[1], N. NEVÁREZ[1], M. SEHRSWEENEY[2], M. WYBRECHT[1], I. K. WOHL[1], E. E. WRIGHT[1], B. J. ARAGONA[1];
[1]Psychology, [2]Ecology and Evolutionary Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: The two distinct families of dopamine (DA) receptor systems within the nucleus accumbens (NAc) shell, the D1- and D2-like receptor systems, differentially mediate the formation of pair bonds in prairie voles. Specifically, activation of D2-like receptors facilitates pair bond formation whereas D1-like activation prevents this behavior. Functionally, however, it

remains unclear how activation in these different receptor systems influence the complex social behaviors important for social bonding. One of the key ways that humans and other mammals engage in social interaction is through vocal communication. In prairie voles, for instance, adult male virgins show a robust increase in the frequency and complexity of ultrasonic vocalizations (USVs) when exposed to novel females, especially if they are in estrous. Here, our objective was to determine whether D2-like activation (i.e. the receptors that promote pair bond formation) promote the production of USVs in male prairie voles when exposed to a novel female, whereas D1-like activation (the receptors that prevent pair bond formation) reduce USV production upon exposure to a female. Specifically, we peripherally administered either a D1-like agonist (SKF 38393) or a D2-like agonist (quinpirole) and quantified changes in USVs upon being exposed to a female. Remarkably, we found that males treated with the D2-like agonist showed both higher rates of USVs, as well as showed a significantly larger repertoire of USV types, compared to males given the D1-like agonist. This is especially significant because, in a separate group of animals, we show that call repertoire size during the first several minutes of exposure to a novel female predicted how strongly the pair eventually bonded and showed associated neuroplasticity within the DA system within the NAc shell. Moreover, follow up choice experiments suggest that females prefer males that have been treated with the D2-like agonist, potentially indicating that adult USVs function to attract mates and that this is mediated in a DA receptor specific manner. Our results strongly suggest that USVs serve to advertise the ability/motivation to form a long-term social bond, making this behavior a key signal in the facilitation of partner choice.

Disclosures: M.L. Gustison: None. **N. Nevárez:** None. **M. Sehrsweeney:** None. **M. Wybrecht:** None. **I.K. Wohl:** None. **E.E. Wright:** None. **B.J. Aragona:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.06/BBB12

Topic: F.01. Neuroethology

Support: Klingenstein Foundation

Title: Temporal dynamics of locus coeruleus activity during courtship in male mice

Authors: D. ECKMEIER, *S. D. SHEA;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Noradrenaline released by the brain stem nucleus locus coeruleus (LC) plays a central role in regulating brain state. Specifically, elevated LC firing is associated with enhanced

arousal, attention, neuronal plasticity and memory. Brain levels of noradrenaline increase during investigation of novel stimuli, in response to pain, during stress, and during social interaction. In the context of reproductive behavior, these surges appear essential for recognition of partners on consecutive encounters. In awake animals, LC neurons exhibit a bimodal firing pattern composed of an arousal-dependent baseline firing rate punctuated by brief bursts in response to stimuli. Whether and how the temporal structure of activity during courtship is distinct from that exhibited during non-social activities are not known. Moreover, while LC was classically thought to respond to novel and arousing stimuli, more recent data show that its firing is more closely locked to initiation of behavior in operant tasks. Whether this temporal relationship to behavior is maintained for social interaction is also not known. We therefore investigated the temporal structure of LC activity and its relationship to interactive behavioral events during courtship in awake male mice. We recorded single unit and multiunit activity using a head-mounted drive with movable 16 channel microwire bundles. We recorded from adult (>3 months) C57/BL6 males during courtship and non-social activities. In addition, we documented behavior with infrared video and ultrasound audio. Preliminary qualitative inspection of the data shows that LC firing is dynamic during courtship of a receptive female partner (e.g. chasing behavior and vocalization). These are the first reported recordings from LC neurons in awake behaving mice freely engaging in social behavior.

Disclosures: D. Eckmeier: None. **S.D. Shea:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.07/BBB13

Topic: G.02. Motivation

Support: NJ Council on Autism

Title: Alterations in USV and cortical structure in KOR-1 KO mice

Authors: B. VILJETIC[1], M. ANSONOFF[1], S. WIJERANTE[1], M. PINTAR[2], M.-R. RASIN[2], *J. E. PINTAR[2];
[1]Neurosci. and Cell Biol., [2]Dept Neurosci & Cell Biol, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: We have analyzed ultrasonic vocalization patterns exhibited by KOR-1 KO mice both as neonates and as young adults. We have found that the number of vocalizations in KOR-1 KO mice are significantly reduced at both stages in different behavioral contexts. Moreover, the

types of vocalizations are also significantly altered in neonates. We have also begun to determine whether any alterations in brain circuitry occur in KO-1 KO mice that potentially could be related to altered behavior. In these initial studies, we have examined cortical organization in 129S6 WT and 129S6 KOR-1 KO as well as compared the 129S6 WT pattern to that of C57Bl6/J mice. Cortical layer organization was assessed using the upper and lower layer markers CDP and TLE4, respectively, while gliogenesis was assessed using APC. Several significant results have arisen from the analysis to date. First, there is a significant decrease in the ratio of upper and lower layer neuronal markers in KOR-1 KO mice compared to isogenic 129S6 WT mice. Second, this change appears to primarily result from a decrease in the number of CDP neurons. Third, there is a dramatic decrease in the ratio of upper to lower layer markers in C57Bl6/J WT mice compared to 129S6 mice. Fourth, the distribution of CDP neurons is restricted to most upper cortical layers in C57WT mice compared to 129S6. Fifth, the extent of glia presence is similar in all three genotypes examined. Together, these data provide the first evidence that neuronal organization within the neocortex is altered not only between distinct mouse strains but also in KOR-1 KO mice.

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Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.08/BBB14

Topic: F.01. Neuroethology

Support: Sao Paulo Research Foundation - FAPESP

Brazilian National Counsel of Technological and Scientific Development - CNPq

Title: Movement and electric analysis of freely swimming pulse type weakly electric fish

Authors: ***R. D. PINTO**, A. C. FREIRES DE OLIVEIRA, R. T. GUARIENTO, L. O. B. ALMEIDA, I. H. Z. DE STEFANI, M. R. GONÇALVES;
Inst. Fisica De Sao Carlos - Univ. Sao Paulo, Sao Carlos, Brazil

Abstract: Weakly electric fish are unique model systems in neuroethology because they allow experimentalists to non-invasively record spatio-temporal electric patterns of pulses used by the central nervous system in electrocommunication and electrolocation [1]. Pulse-type electric fish present stereotyped pulses but are able to dynamically alter their inter pulse intervals (IPIs)

according to different behavioral contexts such as aggression, hiding, mating, as well as when stimulated with different artificial distributions of IPIs [2] as if they were comming from conspecifics.
Here we address how the behavior of fish performing a dominance contest is reflected by electrical patterns and movement, simultaneously recorded. We are specially interested in a behavior in which only one of the fish intermittently replace its pulses by a continuous pattern known as chirp [4]
Our experiments consist in recording simultaneously two video cameras and a 12 electrode array assembled in a big tank (1x0.5x0.5)m, where two *Gymnotus carapo* specimens, previously unknown to each other, are allowed to freely swim and interact.
A dedicated computer algorithm was applied to detect the timing and discriminate fish pulses [3] and another algorithm was applied to infer the 3D position of the fish from the camera's recording.
After the contest, we report the statistics of IPIs of both dominant and submissive fish and correlate them to their movements. We also show that it is possible to extract the position of fish from the electrode array time series. We found that chirping usually occurs either when fish are close to each other or the submissive fish is approached by the dominant. The chirping fish also usually retreats immediately after the chirp.
[1] Bullock TH (1999) J Exp Biol 202: 1455-1458; PMID 10210686
[2] Forlim CG, Pinto RD (2014) PLoS ONE 9(1): e84885. doi:10.1371/journal.pone.0084885
[3] Matias P, Slaets JFW, Pinto RD (2015) Neurocomputing 153, 191-198. doi:10.1016/j.neucom.2014.11.037
[4] Batista G, Zubizarreta L, Perrone R, et al. (2012) *Ethology* 118.4: 398-410. doi:10.1111/j.1439-0310.2012.02022.x

Disclosures: **R.D. Pinto:** None. **A.C. Freires de Oliveira:** None. **R.T. Guariento:** None. **L.O.B. Almeida:** None. **I.H.Z. de Stefani:** None. **M.R. Gonçalves:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

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Program#/Poster#: 444.09/BBB15

Topic: F.01. Neuroethology

Support: FAPESP 2013/09013-8

CNPq

CAPES

Title: Dynamics of electrical behavior of Gymnotus carapo electric fish during dominance contest

Authors: *R. TUMA GUARIENTO[1], T. S. MOSQUEIRO[2], P. MATIAS[3], V. CESARINO[3], L. O. B. ALMEIDA[3], R. D. PINTO[3];
[1]Dept. of Physics and Interdisciplinary Sci., Univ. of Sao Paulo - Sao Carlos Inst. of, Sao Carlos, Brazil; [2]Biocircuits Inst., Univ. of California, San Diego, CA; [3]Dept. of Physics and Interdisciplinary Sci., Univ. of Sao Paulo - Sao Carlos Inst. of Physics, Sao Carlos, Brazil

Abstract: Weakly electric fishes detect a self-generated electric field to detect objects in the environment [1] and to communicate to conspecifics [2]. *Gymnotus carapo* is a pulse-type electric fish that shows a strong non-sex biased dominance relationship [3], which makes this species an interesting model for studying dominance establishment [4]. Recent studies dwelled on how information is electrically communicated during the dominance contest [5]. We hypothesize that electrical behavior has an important role in dominance determination, since fish express very different electrical patterns during and after the dominance contest. Understanding the dynamics of such process can bring new insights of the neuroethology of these animals: how a pulse train mediated interaction can lead to behavioral changes. In this study we analyzed quantitatively how the electrical behavior changes over time using a state-of-art method of pulse-sorting the signals from two freely swimming fish.
One of the main submissive cues is called chirp, when an animal emits a high frequency noise instead of pulses [3]. We detected these regions by using supervised learning, trained with manually selected chirp and non chirp segments. In addition we are also able to detect and sort the emitter of each pulse [6].
During the beginning the contest, we found that both fishes present transitory frequency increases. However, after a few minutes, one of them, the future dominant, establishes a higher average pulse frequency, while the other, the future submissive, begins to chirp and emits pulses with intervals 10 times larger than if it was alone. The number of chirps and offs is proportional to how submissive the fish is, i.e., how likely it was to run away during the contest.
[1] Von der Emde, Gerhard. *Journal of experimental biology* 202.10 (1999): 1205-1215
[2] Kramer, Bernd. Vol. 29. Springer Science & Business Media, 2012
[3] Batista, Gervasio, et al. *Ethology* 118.4 (2012): 398-410
[4] Zubizarreta, Lucía, Philip K. Stoddard, and Ana Silva. *Ethology* 121.1 (2015): 8-16
[5] Mosqueiro, Thiago, et al. *2016 Annual Conference on Information Science and Systems (CISS)*. IEEE, 2016.
[6] Matias, Paulo, Jan Frans Willem Slaets, and Reynaldo Daniel Pinto. *Neurocomputing* 153 (2015): 191-198

Disclosures: R. Tuma Guariento: None. **T.S. Mosqueiro:** None. **P. Matias:** None. **V. Cesarino:** None. **L.O.B. Almeida:** None. **R.D. Pinto:** None.

Poster

444. Social Communication in Non-Avian Models

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Program#/Poster#: 444.10/BBB16

Topic: F.01. Neuroethology

Support: Sao Paulo Research Foundation - FAPESP. Grant: 2014/23119-6

Brazilian National Counsel of Technological and Scientific Development - CNPq

Title: Non-invasive inference of neuronal refractory time in pulse type weakly electric fish communication

Authors: *A. S. RIOS, R. T. GUARIENTO, L. O. B. ALMEIDA, I. H. Z. DE STEFANI, R. D. PINTO;
Dept. of Physics and Interdisciplinary Sci., Univ. of Sao Paulo, Sao Carlos, Brazil

Abstract: Electrolocalization and electrocommunication are complex tasks performed by the nervous system of weakly electric fish [1]. Pulse type fish process information from a myriad of electric field sensors disposed over their skin to sense self- and conspecific- stereotyped electric pulses, called electric organ discharges (EOD). These remarkable animals are able to change the complexity of their spatio-temporal electric patterns according to different stimuli [2]. In their central nervous system, the sensorial information reach a population of neurons (spherical cells) dedicated to fire when a pulse is detected, and they seem to be preferentially tuned to self generated pulses [3]. Here, we address the role of such refractory time in electrocommunication. In principle, a long refractory time poses a blind period after a self EOD in which no pulses from other conspecifics can be detected. We show that using some tools from Information Theory we were able to access such refractory times noninvasively. Our experiment consists in recording long time series from a 12 electrode array assembled in a big tank (1x0.5x0.5)m where two *Gymnotus carapo* specimens are allowed to freely swim and interact [2]. A dedicated computer algorithm was applied to detect the timing (time stamp) and discriminate fish pulses [4]. We compute the Transfer Entropy (TE) and the Average Mutual Information (AMI) to infer the amount of information that one fish's EOD time stamps express about the other fish's EODs time stamps [2,5] in several time windows. We chose one of the fish as the response fish and, for each value of a response refractory time parameter (*Tr*), we re-sample the stimuli fish EODs by removing all those EODs that occurred during *Tr* after a pulse in the response. Then we compute TE and AMI for the re-sampled series. We found peaks of both TE and AMI in the same windows along the series for values of *Tr* that are compatible with the refractory time of the spherical cells measured *in vitro*. Our result is an independent evidence that such refractory times must be considered in communication analysis.
[1] Bullock TH, Hopkins CD, Fay RR, eds. (2006) Electroreception, Springer.

[2] Forlim CG, Pinto RD (2014) PLoS ONE 9(1): e84885. doi:10.1371/journal.pone.
[3] Nogueira J, Caputi AA (2011) PLoS ONE 6(7): e22159. doi:10.1371/journal.pone.
[4] Matias P, Slaets JFW, Pinto RD (2015) Neurocomputing 153, 191-198. doi:10.1016/j.neucom.2014.11.
[5] Ito S, Hansen ME, Heiland R, Lumsdaine A, Litke AM, et al. (2011) PLoS ONE 6(11): e27431. doi:10.1371/journal.pone.

Disclosures: **A.S. Rios:** None. **R.T. Guariento:** None. **L.O.B. Almeida:** None. **I.H.Z. De Stefani:** None. **R.D. Pinto:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.11/DP06 (Dynamic Poster)

Topic: F.01. Neuroethology

Support: NSF Career Grant IOS-1054578-007

McKnight Foundation Scholar Award

Title: Specification of male versus female acoustic communication behaviors in *Drosophila* virilis

Authors: *C. A. BAKER, X.-J. GUAN, M. MURTHY;
Neurosci., Princeton Univ., Princeton, NJ

Abstract: Many animals, including insects, engage in acoustic duetting, a complex behavior in which males and females control the timing of their courtship songs relative to each other. However, the mechanisms underlying duetting remain largely unknown. Our lab has recently developed *Drosophila virilis* as a model for studying acoustic duetting. Male and female *D. virilis* produce spectrotemporally distinct songs via wing vibration; males coordinate their songs with females based on auditory feedback, while females rely largely on tactile feedback to time their songs (LaRue et al 2015, *eLife* 4:e07277). These differences raise the question of how the underlying circuits produce these sexually dimorphic behaviors. Here we test whether the *fruitless* gene, which plays a critical role in directing male-specific courtship behaviors in other insects, contributes to sex differences in *D. virilis* courtship.
In *Drosophila melanogaster*, only males sing during courtship. Particular *fruitless* transcripts are sex-specifically spliced, leading to the production of FruM protein in male *D. melanogaster* neurons. The presence of the Transfomer protein in females prevents FruM expression by

directing female splicing of the sex-specific transcripts. When female *D. melanogaster* are forced to express FruM, they court wild-type females and sing (Demir & Dickson 2005, *Cell* 121:785-794), albeit with an aberrant song (Clyne & Miesenboch 2008, *Cell* 133:354-363).
To test whether *fruitless* also contributes to *D. virilis* courtship behaviors, we used CRISPR-Cas9 to remove the Transformer binding sites from the *fruitless* gene, thus forcing production of the male-specific FruM protein in both sexes. We recorded song and associated courtship behaviors in high throughput and used single-fly PCR to determine which flies carried our designed mutation.
We confirmed that FruM transcripts were made in mutant female brains. Unlike *D. melanogaster*, FruM-expressing *D. virilis* females did not court wild-type females. *D. virilis* FruM females duetted with males but produced altered song parameters. Mutant females were also less receptive and less fertile, despite vigorous courting by males. Finally, *D. virilis* FruM females engaged in aggressive interactions seen in wild-type male-male, but not male-female, pairings. Our results reveal that *fruitless* is indeed involved in sex-specific behaviors in *D. virilis*, but its role in this duetting species may be different from that in *D. melanogaster.* Further experiments will test this prediction, as well as generate tools to label and activate/silence *fruitless*-expressing neurons in *D. virilis* to dissect the role these neurons play in acoustic duetting.

Disclosures: C.A. Baker: None. **X. Guan:** None. **M. Murthy:** None.

Poster

444. Social Communication in Non-Avian Models

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Program#/Poster#: 444.12/BBB17

Topic: F.01. Neuroethology

Title: A circuit screen for song production neurons in *Drosophila melanogaster*

Authors: *A. HAMMONS[1,2], D. PACHECO[2], M. MURTHY[2];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ

Abstract: The courtship song production circuit in the fruit fly *Drosophila melanogaster* provides an excellent animal model for studying the decision-making and motor pattern generation that are important for mate selection in a number of species. With the small and genetically tractable nervous system of *Drosophila*, identification of the specific neurons involved in courtship song production allows interrogation of the principles underlying the transformation of complex patterns in the nervous system to a motor output. Though some neural classes in the song pathway have been identified (von Philipsborn et al. Shirangi et al.), there are

likely many more neurons in these circuits. Moreover, the functional properties and connectivity of identified neuronal classes remains uncharacterized. To uncover additional components of the neural circuit underlying song production, we performed a neural activation screen for ventral nerve cord (VNC) neurons that drive song production in headless males by expressing the temperature-sensitive ion channel TrpA1 in subsets of neurons (labeled by the Janelia GAL4 collection). We identified seven GAL4 lines capable of song production when artificially activated. An intersectional approach where TrpA1 was restricted to neurons expressing either the *fruitless* or *doublesex* gene revealed that five of these GAL4 lines contain VNC song production neurons that do not express either of these two genes. Chronic inactivation (using tetanus toxin light-chain (TNT), a genetically encoded protein that blocks synaptic transmission) of these candidate neurons during a courtship pair assay that measures both male and female movement while also recording song, allowed us to characterize these neurons' effect on song patterning and female responses to song. Ongoing experiments using *in vivo* calcium imaging of song circuit neurons in the VNC combined with optogenetic activation of P1 song command neurons will determine whether or not these newly identified neurons form an interconnected song circuit with those neurons previously identified.

Disclosures: A. Hammons: None. **D. Pacheco:** None. **M. Murthy:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

Support: NSF career award

Alfred P Sloan Foundation

Human Frontier Science Program

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Title: Characterizing neural activity in the song motor circuit of *Drosophila*

Authors: *D. A. PACHECO PINEDO, M. MURTHY;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: *Drosophila melanogaster* exhibit a robust and complex courtship ritual, during which males chase females and produce a courtship song by unilateral wing vibration. This acoustic signal is arranged in bouts that consist of two syllables: sine and pulse. Drosophila singing is actively patterned in response to sensory feedback from the female (Coen et al. 2014). Recent mapping of the neural centers underlying song production have identified clusters of neurons located in the brain and the ventral nerve cord (von Philipsborn et al; Shirangi et al.). Despite the characterization of some components of the song circuit, we still don't fully understand how this patterned behavior is generated and how sensory cues are integrated to modulate song features. This is partially because many neurons of the song motor circuit remain to be mapped and none of these neurons have been functionally characterized. In order to have a singing-related activity map with a fine anatomical resolution, we have developed an assay that combines stimulation of different stages of the song circuit, monitoring of neural activity throughout the entire nervous system, along with a readout of fictive singing in a fixed fly. The Gal4-UAS binary system was used to express a red-shifted channel rhodopsin (ReachR) in specific components of the song circuit. A second and orthogonal binary system, LexA-LexAop, was used to independently express a genetically encoded calcium indicator (GCAMP6s) broadly. We developed a whole brain and nerve cord 3D-imaging pipeline that automatically segments regions of interest and maps them to reference brain coordinates for systematic anatomical comparison across flies. As fly dissections prevent recordings of song production directly, we extra-cellularly recorded from the motor neurons that drive the wing muscles, providing a fictive readout of singing. We present data on the neural dynamics and functional connectivity that underlie song production and patterning in *Drosophila*.

Disclosures: D.A. Pacheco pinedo: None. **M. Murthy:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.14/BBB19

Topic: F.01. Neuroethology

Support: NIH R01 DC012087

Title: Social consequences of interruptions during marmoset conversations

Authors: *C. TOARMINO[1], C. T. MILLER[2];
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Abstract: Social living is an integral feature of primate societies. As such, social rules emerge that regulate individual and group behaviors. The ability of group members to recognize and follow these rules is critical to their survival and reproductive success, and violations to these rules may have a significant behavioral impact. Common marmosets, like other primates, follow rules during social and communicative interactions. However, it is unknown how violations to these rules impact the communicative exchange and the social treatment of the violator. Here, we examined the social consequences of interruptions during their natural conversations called antiphonal calling. We used a novel, multi-speaker interactive playback design with two 'Virtual Monkeys' (VMs) that simulated individual marmosets to test how interruptions impacted the dynamics of the conversation. One VM was denoted VM-Norm, as it produced normal responses to subjects' vocalizations, and the other was denoted VM-Int, as it occasionally interrupted subjects' own phee calls. Two types of phee calls were produced by the VMs: 1) antiphonal phee calls were broadcast in response to subjects' vocalizations, and 2) when subjects were vocally inactive for a period of time, one VM was selected to broadcast a spontaneous phee call to attempt to engage the subject in communication. This design afforded subjects the opportunity to learn about the individual behaviors of each VM and make decisions about whether or not to interact with those VMs based on that information. We recorded subjects' responses to each VM's different phee call types. We found that subjects were significantly less likely and slower to respond to an interruption from VM-Int than an antiphonal call from VM-Int or VM-Norm. Interruptions effectively ended the vocal exchange between subjects and VM-Int for that bout of calling. However, interruptions had no effect on subjects' decisions to respond to either VM when the VM initiated the vocal exchange (*i.e.,* a VM's spontaneous call). We repeated this experiment with familiar and non-familiar stimuli, different age groups, and an increased rate of interrupting and found that these results persist regardless of these conditions. We are currently examining how interacting with the same interrupter over multiple days influences subjects' decisions in conversations.

Disclosures: C. Toarmino: None. **C.T. Miller:** None.

Poster

444. Social Communication in Non-Avian Models

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Program#/Poster#: 444.15/BBB20

Topic: F.01. Neuroethology

Support: NIH R01 DC-012087

Title: Primate frontal cortex neurons predict the outcome of natural conversations

Authors: *V. JOVANOVIC[1], S. NUMMELA[1], L. DE LA MOTHE[2], C. T. MILLER[1];
[1]UCSD, San Diego, CA; [2]Dept. of Psychology, Tennessee State Univ., Nashville, TN

Abstract: Communication is an inherently interactive process that weaves the fabric of society together for both human and nonhuman primates alike. Yet much of what is known about the neural basis of communication in our simian cousins has been limited to studies of static signals. To investigate the properties of the primate brain during active social signaling, we recorded the activity of frontal cortex neurons as freely-moving marmosets engaged in natural conversations. The responses of all neurons (n=258) to vocalization stimuli were compared in two behavioral contexts during conversations with a Virtual Marmoset (VM): when subjects produced a response following hearing a vocalization stimulus (Antiphonal) and when no response occurred (Independent). Across three subjects, PCA indicated that the greatest source of variance in firing rate was explained by these two behavioral contexts. Furthermore, we observed that frontal cortex activity could reliably predict whether a conspecific's vocalization would elicit a vocal response with 92% accuracy. In fact, correct classification was not stimulus-driven, as neural activity preceding the stimulus could still reliably predict the behavioral outcome. This finding was surprising for at least the following two reasons. First, this population of neurons did not exhibit robust changes in firing rate to vocalization stimuli. Second, the PCA analysis included all neurons, not just those that were modulated by the conversational behavior. These data suggest that the state of the brain prior to hearing a conspecific vocalization determines whether marmosets will produce a response and engage in a conversation. The close coupling between brain state and behavioral context is further evidenced by analyses of extended conversations, as brain and behavior exhibit a near perfect correlation. These data suggest that active social signaling may have a profound effect on the neuronal processes and lend unique insight into facets of the primate social brain.

Disclosures: V. Jovanovic: None. **S. Nummela:** None. **L. de la Mothe:** None. **C.T. Miller:** None.

Poster

444. Social Communication in Non-Avian Models

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Topic: F.01. Neuroethology

Support: Poitras Foundation

Stanley Center

Broad Institute

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McGovern Institute

Title: Asymmetry in vocal communication in marmosets - influence of social context and gender differences

Authors: *J. SHARMA[1], R. LANDMAN[2], J. HYMAN[3], L. BRATTAIN[4], K. JOHNSON[4], T. QUATIERI[4], K. SRINIVASAN[3], A. WISLER[4], G. FENG[5], M. SUR[6], R. DESIMONE[3]; [1]Picower Inst. For Learning & Memory, MIT and MGH, Cambridge, MA; [2]Broad Inst. and McGovern Inst., Cambridge, MA; [3]McGovern Inst. of Brain Res., Cambridge, MA; [4]Lincoln Labs, MIT, Lexington, MA; [5]McGovern Inst. of Brain Res. and Broad Inst., Cambridge, MA; [6]Picower Inst. for Learning and Memory and Simons Ctr. for Social Brain, Cambridge, MA

Abstract: Common marmosets are highly gregarious animals and use a rich vocal repertoire while communicating with conspecifics during social interactions. Recent studies indicate that they take turns in uttering calls (anti-phonal calling). There is also evidence that these exchanges are modulated by social context and specific calls are used to locate a group member when out of visual contact, when under threat or to show anxiety. To study these vocal/social interactions in a naturalistic home-cage environment, we developed a wireless lightweight flexible neck collar, equipped with a micro-electro-mechanical system (MEMS) acoustic microphone, a non-acoustic contact microphone for detecting caller vocal-fold vibration, and a Bluetooth module for wireless data transmission. The initial testing was done on marmoset dyads and spectral analysis of their calls was performed to identify individual caller. Approximately 80% of calls could be attributed to an individual based on relative sound pressure alone. The remaining 20% were attributed with addition of data from the contact microphone. Cross-correlation between audio channel and vibrational signal allows identification of most likely caller. We examined vocal interactions between dyads within the home-cage environment. We find that antiphonal calling occurs not only for ‘phee’-calls, but also for other calls such as ‘trill’, and even while in visual contact. We selectively removed one member of a dyadic pair (male or a female) from the home cage for short periods, while within or without visual contact. Analysis shows that there is directionality in these interactions - when the female is out of the home cage but within view, there is an increase in temporal coherence, where one animal calls at a lag of about 0.5 sec after his/her partner. An asymmetry between dyads is also found when one animal is taken out of the room, but within audible range. When the female is taken out, dyads ‘phee’-call back and forth, but when the male is taken out, there is no ‘phee’-calling. The first 'phee'-call is typically uttered by the animal that is outside. Directionality in vocal interactions may be thus be associated with the sex of the animal, social context, dominance and relatedness. Further research is underway on multiple dyads to confim and to explore neural underpinnings and behavioral consequences of this asymmetry.

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Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.17/BBB22

Topic: F.01. Neuroethology

Title: A framework for studying the neuronal basis of interactive social behavior

Authors: ***K. HAROUSH**[1], Z. WILLIAMS[2];
[1]Neurosurg., [2]Harvard Med. Sch., Boston, MA

Abstract: Social interactions are a fundamental part of many animal societies, and are increasingly more elaborated and sophisticated in humans and non-human primates. A key aspect of successful social interchange is the ability of individuals to cooperatively interact in order to reach mutually favorable goals such as obtaining and sharing food, defending from predators and building social bonds that benefit future generations. In human societies, cooperation further plays a central role in many interpersonal, economic and political decisions. At the same time, many mental health disorders are characterized by shared substantial deficits in social interactions. Therefore, our ability to understand and treat these various disorders depends on our ability to crack the neuronal coding of basic elements of social interactions in healthy individuals. However, the single neuronal basis, network computations and causal underpinnings of social interactions is largely unknown. A main challenge remains decomposing this complex behavior into a biological problem that could be quantitatively studied. We propose a framework for studying social interactive behavior by using game-theory driven principles. This formal framework allows disentangling neuronal signals that relate to the multiple aspects of social interactions, such as predicting another individual's intentions, weighing previous experiences with others and assessing possible personal profit.

Disclosures: **K. Haroush:** None. **Z. Williams:** None.

Poster

444. Social Communication in Non-Avian Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 444.18/BBB23

Topic: F.01. Neuroethology

Support: Max Planck Research Group

Title: Understanding language genetics: establishing bats as a mammalian model of vocal learning

Authors: P. RODENAS-CUADRADO[1], J. MENGEDE[1], M. YARTSEV[2], U. FIRZLAFF[3], *S. VERNES[1,4];
[1]Max Planck Inst. For Psycholinguistics, Nijmegen, Netherlands; [2]Helen Wills Neurosci. Inst., Berkeley, CA; [3]Lehrstuhl für Zoologie, Freising-Weihenstephan, Germany; [4]Donders Ctr. for Cognitive Neuroimaging, Nijmegen, Netherlands

Abstract: The capacity for speech and language is a fundamental trait of humankind, and is of intense interest across diverse fields including linguistics, anthropology, neurobiology, cognitive neuroscience and molecular and evolutionary biology. Although humans are the only extant species with language, animal models have begun to reveal the biological bases of language relevant traits like vocal learning. Vocal learning, the ability to acquire and reproduce sounds through vocal imitation is essential for the acquisition of spoken language and is an extremely rare ability, found only in humans and a handful of other species (some birds, bats, elephants, whales and seals). To date, the only neuro-genetic studies on vocal learning have been performed in songbirds. Although these studies have given important insights into how genes may contribute to vocal learning, a comparative approach with other species will be vital in order to obtain a complete picture on how vocal learning is genetically encoded.
To this end, we are establishing bats as a molecular model system for the study of vocal learning. Bats are able to employ an astonishingly complex vocal repertoire for conveying social information and there is evidence across multiple bat species for vocal learning. As mammals, they are evolutionarily distant from songbirds and thus provide an ideal comparison for findings from avian systems. We present a range of work including gene knockdown studies, brain expression analysis, immediate early gene staining and transcriptomics, aimed at establishing bats as a viable molecular model for the study of vocal learning. Taken together these studies will ultimately advance our knowledge about the molecular encoding of vocal learning in the mammalian brain. Moreover this work will be highly complementary to work in avian models. Given the evolutionary distance between bats (mammalia) and songbirds (aves) this presents a unique opportunity to compare how vocal learning has evolved across these very different species. Integrating our findings in bats with songbird data and clinical studies in humans will

provide fundamental insights into the genetic underpinnings of vocal learning and ultimately shed light on how human spoken language may have evolved.

Disclosures: **P. Rodenas-Cuadrado:** None. **J. Mengede:** None. **M. Yartsev:** None. **U. Firzlaff:** None. **S. Vernes:** None.

Poster

444. Social Communication in Non-Avian Models

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Program#/Poster#: 444.19/BBB24

Topic: F.01. Neuroethology

Support: NSFC 31372217

Title: Changes in EEG approximate entropy reflect auditory processing and functional complexity in frogs

Authors: ***G. FANG**, Y. LIU, Y. TANG;
Chengdu Inst. of Biology, CAS, Sichuan, China

Abstract: Brain systems engage in what are generally considered to be among the most complex forms of information processing. In the present study, we investigated the functional complexity of anuran auditory processing using the approximate entropy (ApEn) protocol for electroencephalogram (EEG) recordings from the forebrain and midbrain while male and female music frogs (*Babina daunchina*) listened to acoustic stimuli whose biological significance varied. The stimuli used were synthesized white noise (reflecting a novel signal), conspecific male advertisement calls with either high or low sexual attractiveness (reflecting sexual selection) and silence (reflecting a baseline). The results showed that 1) ApEn evoked by conspecific calls exceeded ApEn evoked by synthesized white noise in the left mesencephalon indicating this structure plays a critical role in processing acoustic signals with biological significance; 2) ApEn in the mesencephalon was significantly higher than for the telencephalon, consistent with the fact that the anuran midbrain contains a large well-organized auditory nucleus (torus semicircularis) while the forebrain does not; 3) for females ApEn in the mesencephalon was significantly different than that of males, suggesting that males and females process biological stimuli related to mate choice differently.

Disclosures: **G. Fang:** None. **Y. Liu:** None. **Y. Tang:** None.

Poster

444. Social Communication in Non-Avian Models

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Topic: F.01. Neuroethology

Support: NSF Grant IOS-1146501

USTAR startup fund

Title: Call initiation in African clawed frogs

Authors: *A. YAMAGUCHI;
Biol., Univ. of Utah, Salt Lake City, UT

Abstract: The most salient product of brain activity is behavior. Understanding the neural mechanisms underlying behavior presents a formidable challenge requiring a well-chosen model system. Vocalizations of the African clawed frog *Xenopus laevis* are an exceptionally well suited model system for this objective for the following reasons. First, a simplified mechanism of vocal production (independent of respiratory musculature) allows straightforward interpretations of neuronal activity with respect to behavior. Second, neural mechanisms of calling can be studied *in vitro*: application of serotonin (5-HT) to an isolated male brains readily elicits fictive advertisement calls. Using this "singing brain in a dish" preparation, we have identified and characterized the basic building blocks of *Xenopus* vocal circuits to date. Although we have been successful in identifying the neurons that are involved in rhythm generations, we have very little understanding of initiation of this vocal circuitry in the brainstem. The goal of the present study is to identify a population of neurons that mediate call initiation. Using local field potential (LFP) recordings, we discovered a population of neurons in the regions medial to the anterior laryngeal motor nucleus (n.IX-X) that show stereotyped activities prior to call initiation. The activity recorded in these regions are rhythmic (~ 35Hz) and precede the beginning of calls by ~100 msec. This activity are blocked by antagonists for synaptic transmission (50uM APV, 10uM DNQX, 10uM bicuculline, and 1uM strychnine), indicating that it is not derived from electrically-coupled pacemaker neurons . It also is not mediated by vocal motor neurons because when all the laryngeal motoneurons are silenced by retrograde loading of QX-314, an intracellular Na+ channel blocker, via the laryngeal motor axons, rhythmic activity can still be elicited by serotonin application. Thus, we conclude that there is a population of synaptically connected interneurons medial to the anterior n.IX-X that play a role in call initiation in response to 5-HT in the *Xenopus* brainstem. At a molecular level, we have previously shown that call initiation is mediated by 5-HT_{2C} receptors, which are expressed in rostral raphe nucleus. Because of the proximity of the LFP recording site and the rostral raphe nucleus, we suggest that a

population of interneurons in the rostral raphe nucleus generate oscillatory rhythms in response to 5-HT and activate the vocal pattern generator in the brainstem.

Disclosures: A. Yamaguchi: None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 445.01/BBB26

Topic: F.05. Neuroimmunology

Title: Innate lymphoid cells: novel mediators of gut-brain immune regulation?

Authors: *N. C. DERECKI[1], G. ALEMAN-MUENCH[2], P. SOROOSH[2], H. BANIE[2], J. KARRAS[2], L. CHANG[2], M. HESSE[2], T. LOVENBERG[1], A. BHATTACHARYA[1]; [1]Neurosci., [2]Immunol., Janssen Res. and Develop., San Diego, CA

Abstract: Recent clinical data strongly suggests that mood disorders of the central nervous system (CNS), including major depressive disorder (MDD) are associated with increased levels of peripheral chronic inflammation. Along these lines, comorbidity between Inflammatory Bowel Disease (IBD) and MDD have been documented in epidemiological studies. The precise cellular and molecular mechanisms, however, that underlie these connections between peripheral gut inflammation and CNS dysfunction have remained elusive. To probe the hypothesis that players involved in gut inflammation may be linked with concomitant neuroinflammation, we examined CNS tissues in parallel with intestinal tissues in the innate immune anti-CD40 mouse model of IBD. Flow cytometric examination of peripheral tissues (colon and blood) in parallel with CNS tissues (meningeal lymphatics and brain) revealed a robust, synchronous response by meningeal macrophages and CNS microglia to induction of intestinal pathology. In addition, infiltration of brain by significant numbers of peripheral monocytes and granulocytes was observed. Finally, an unexpectedly prominent population of several subtypes of innate lymphoid cells (ILC) were revealed to be present in meningeal tissues; moreover, these cells were dynamically regulated as a result of peripheral anti-CD40-induced inflammation. Phenotypic characterization of these meningeal ILC revealed them to exhibit basic properties consistent with ILC in other tissues but significantly different (from gut and lung ILC) in terms of levels of receptor and transcription factor expression. In line with work examining the effects of IL-23, IL-25, and IL-33 on ILC, we saw robust local CNS expansion following peripheral administration of these cytokines. Interestingly, parenchymal microglia and myeloid cells in the meningeal spaces were also significantly different in terms of activation phenotype and cytokine production in $RAG2^{-/-}$ mice—which lack T and B cells—and $RAG2^{-/-}$Common $\gamma C^{-/-}$ mice—

which lack T and B cells and ILC, suggesting a key role for ILC in immune regulation of the CNS. These findings reveal a novel network of immune cells that may be involved in both homeostatic regulation of peri-CNS areas and locally-mediated dysregulation of these same areas during peripheral inflammation.

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Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

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Program#/Poster#: 445.02/CCC1

Topic: F.05. Neuroimmunology

Support: NIH NIGMS R01GM089807

NIH NIGMS R01GM057226

Title: Cholinergic signalling in forebrain regulates peripheral inflammation

Authors: ***V. A. PAVLOV**[1,2], K. R. LEHNER[2], H. A. SILVERMAN[3,2], M. ADDORISIO[3], T. TSAAVA[3], M. OCHANI[3], W. HANES[3], P. S. OLOFSSON[3], S. S. CHAVAN[3], N. M. NATHANSON[5], Y. AL-ABED[4], V. F. PRADO[6], M. A. M. PRADO[6], K. J. TRACEY[3]; [1]Ctr. for Bioelectronic Med., The Feinstein Inst. For Med. Res., Manhasset, NY; [2]Hofstra Northwell Sch. of Med. at Hofstra Univ., Hempstead, NY; [3]Ctr. for Biomed. Sci., [4]Ctr. for Mol. Innovation, The Feinstein Inst. for Med. Res., Manhasset, NY; [5]Dept. of Pharmacol., Univ. of Washington, Seattle, WA; [6]Dept. of Physiol. and Pharmacology, Dept. of Anat. & Cell Biol., Robarts Res. Institute, The Univ. of Western Ontario, London, ON, Canada

Abstract: The brain regulation of physiological functions is integral to survival**.** Relatively little is known about the brain neuronal regulation of immune function and inflammation. Brain cholinergic signals have been implicated in controlling peripheral inflammation (Nat Rev Endocrinol, 2012, 8:743), but specific insight into the role of the inherently complex system of neurons releasing acetylcholine and cholinergic receptors in the brain is lacking. The basal forebrain cholinergic system is a major neuromodulatory system in the brain. Here, by using neuron- and receptor-specific genetic and pharmacological approaches, we studied the role of forebrain cholinergic signalling in the regulation of peripheral inflammation and the mediating role of the M1 muscarinic acetylcholine receptor (M1 mAChR). Selective elimination of

acetylcholine release in the forebrain by genetic Cre/loxP ablation of the vesicular acetylcholine transporter (a protein required for synaptic acetylcholine release) and interruption in vagus nerve activity abolished the suppression of serum TNF by the centrally-acting cholinergic drug galantamine in murine endotoxemia. Optogenetic stimulation of basal forebrain medial septum cholinergic neurons, a major source of brain cholinergic input to areas with abundant M1 mAChR localization, reduced serum TNF in endotoxemic mice, as compared to sham stimulation ($P<0.04$). Activation of acetylcholine action on the brain M1 mAChR by using intracerebroventricular administration of the selective positive allosteric modulator benzyl quinolone carboxylic acid (BQCA) also suppressed serum TNF levels ($P<0.02$). In addition, peripheral administration of the centrally-acting BQCA suppressed serum TNF and improved survival in lethal murine endotoxemia and polymicrobial sepsis and these effects were abolished in M1 mAChR KO mice. These findings provide novel insights into the role of forebrain cholinergic signalling in the regulation of innate immune responses and inflammation and are of interest for developing new brain-based treatments for conditions characterized by excessive pro-inflammatory cytokine levels and inflammation.

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Poster

445. Neuroimmunology: Regulating Systems

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Program#/Poster#: 445.03/CCC2

Topic: F.05. Neuroimmunology

Support: NIH 1P01 AI073693

Title: Antibody efflux from cerebrospinal fluid to systemic circulation

Authors: ***J. NESTOR**, C. KOWAL, B. VOLPE, B. DIAMOND;
Ctr. for Autoimmune and Musculoskeletal Dis., Feinstein Inst. For Med. Res., Manhasset, NY

Abstract: Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) is a disorder seen frequently in Systemic Lupus Erythematosus (SLE) patients, which covers a wide range of symptoms such as stroke, cognitive dysfunction and depression. Our lab believes that some of these symptoms are mediated by autoreactive antibodies, which have cross-reactivity to both dsDNA and the NMDA receptor. We have previously shown that these antibodies can cause

damage in the brain parenchyma, when different agents are used to disrupt the blood brain barrier (BBB). In depth studies have been completed looking at the events causing breaches in the BBB, but until recently with the discovery of antibody transportation through the "glymphatic system" and perivascular spaces, the mechanisms determining antibody efflux from the CNS have remained elusive. This study addressed the flow of antibodies from the CSF into either the brain parenchyma or back into systemic circulation. Our model involves injection of antibody and control proteins directly into the lateral ventricle of mice and measuring corresponding protein levels in both brain parenchyma and serum. Our study suggests an IgG-specific transport mechanism mediated by FcRn, whereas the efflux of other antibody classes and non-immunoglobulin proteins appeared to be unaffected by the presence of the FcRn receptor. Understanding the movement of antibodies from the CSF into different areas of the brain can help us understand the different presentations associated with NPSLE and other antibody-mediated neuropsychiatric disorders. The efflux of antibodies from the CSF back into circulation will also be important in the development, design and dosing of new antibody-based treatments that are being used to target various neurological disorders.

Disclosures: **J. Nestor:** None. **C. Kowal:** None. **B. Volpe:** None. **B. Diamond:** None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 445.04/CCC3

Topic: F.05. Neuroimmunology

Title: The involvement of the brain's choroid plexus in stress response

Authors: *A. KERTSER, K. BARUCH, M. SCHWARTZ;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Severely stressful conditions can trigger an inflammatory response within the brain, yet the underlying mechanism and the implications of this response to post traumatic pathologies are not fully understood. Recently, we identified the brain's choroid plexus (CP) as an active immunological interface between the brain and the circulation, which can support the recruitment of "healing" immune cells to the central nervous system (CNS) following injury. Accordingly, we hypothesized that post traumatic complications associated with cognitive impairments, might involve CP dysfunction as a gateway for leukocyte trafficking. We found that CP gateway activity can be induced in an epithelial NFκB-dependent mechanism, which could be experimentally suppressed, using endogenous inhibitors. Addressing this signaling pathway in a mouse model, we found that glucocorticoids can directly affect CP gateway activity

and may lead to reduced CNS immunosurveillance following severe traumatic stress. Together, these findings extend our understanding of signals which regulate CP gateway activity, and may point to novel therapeutic targets in treating CNS pathologies.

Disclosures: A. Kertser: None. **K. Baruch:** None. **M. Schwartz:** None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

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Program#/Poster#: 445.05/CCC4

Topic: F.05. Neuroimmunology

Title: Investigating neuroimmune function throughout pregnancy and the postpartum period.

Authors: *M. L. SHERER[1], C. K. POSILLICO[2], J. M. SCHWARZ[2];
[1]Psychology, [2]Univ. of Delaware, Newark, DE

Abstract: It is well known that the maternal immune system changes dramatically during pregnancy in order to prevent the developing fetus from being attacked by the maternal immune system. The reduced activity of many peripheral immune cells combined with an increased production of anti-inflammatory cytokines provides an immune environment very different from a non-pregnant female. As an example, many women who suffer from autoimmune disorders actually find relief from the severity of their symptoms while pregnant due to these changes in immune function. However, these peripheral immune alterations leave the mom at a greater risk for diseases normally mitigated by an inflammatory response. Despite this evidence, no one has examined whether changes in neuroimmune function also occur during pregnancy and the postpartum period. It is well known that changes in immune function are often linked to the onset of certain mental health disorders, including depression. Thus, we hypothesize that changes in immune function that are associated with pregnancy and the postpartum period may increase the risk of postpartum depression following birth. Previous studies in our lab have found significant changes in inflammatory molecules within both the prefrontal cortex and the hippocampus of postpartum rats. In the current study, we investigated the impact of an immune challenge (LPS) during various time points throughout pregnancy and postpartum period on the expression of immune molecules in the brain with future experiments examining how these changes in immune function may influence mood and anxiety during pregnancy and the postpartum period.

Disclosures: M.L. Sherer: None. **C.K. Posillico:** None. **J.M. Schwarz:** None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 445.06/CCC5

Topic: F.05. Neuroimmunology

Title: Three-dimensional mapping of neural-immune interactions in the spleen of naïve mice.

Authors: E. HAMMOND, I. BRUST-MASCHER, E. N. MILLER, *C. REARDON;
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Abstract: Background: The neuro-inflammatory reflex is an important physiologic mechanism for regulating systemic inflammation during both homeostasis and inflammatory diseases. It has previously been shown that the neuro-inflammatory reflex requires interaction between the splenic nerve and choline acetyltransferase (ChAT) expressing T cells. Although it has been suggested that the tyrosine hydroxylase (TH) expressing sympathetic nerves of the spleen communicate with $ChAT^+$ immune cells via synapse formation, alternative mechanisms have not been eliminated.
Objective: To elucidate the nature of the interaction between the sympathetic nerves of the spleen and $ChAT^+$ immune cells.
Methods: Spleens from naïve ChAT-GFP mice were optically clarified using the CLARITY technique and subjected to immunostaining using anti-GFP and anti-TH antibodies, followed by detection with Alexafluor-488 and -546 labelled secondary antibodies. Volumes of tissues (3x3x1.5mm) were acquired using two-photon microscopy. ChAT-GFP^+ splenocytes and TH^+ neurons in the imaged 3D volume were identified and modelled allowing distance between immune cells and neurons to be calculated using Imaris image analysis software. Analysis of B- or T-cells intimately associated with TH^+ neurons was performed in tissue sections using super-resolution Stimulated Emission Depletion (STED) microscopy
Results: In spleens from naïve ChAT-GFP mice we found the vast majority of ChAT-GFP^+ cells were not closely associated with TH+ sympathetic neurons. In calculating the distance to GFP^+ cells to nearest TH^+ neuron, approximately 1% of ChAT-GFP expressing cells were within 5µm of TH^+ nerve fibers. Analysis of closely juxtaposed TH^+ neurons and ChAT-GFP^+ or ChAT-GFP- B-cells or T-cells revealed no difference in the surface area of the 3D contact occurring between neurons and these immune cells.
Conclusions: Our data suggest that the vast majority of ChAT-GFP^+ splenocytes are not intimately associated with sympathetic nerves, and that ChAT-GFP^+ immune cells do not appear to make unique synapse like structures with neurons. These data suggest the existence of an alternative mechanism of neuro-immune communiation.

Disclosures: **E. Hammond:** None. **I. Brust-Mascher:** None. **E.N. Miller:** None. **C. Reardon:** None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

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Program#/Poster#: 445.07/CCC6

Topic: F.05. Neuroimmunology

Title: Increased oxytocin immunoreactivity in male and female germ-free Swiss-Webster mice.

Authors: ***N. V. PETERS**[1], M. J. PAUL[3], B. CHASSAING[2], J. DUNN[1], A. T. GEWIRTZ[2], G. J. DE VRIES[1];
[1]Neurosci. Inst., [2]Ctr. for Inflammation, Immunity and Infection, Georgia State Univ., Atlanta, GA; [3]Psychology, Univ. at Buffalo, Buffalo, NY

Abstract: The microbiota is the collection of microorganisms that inhabit our bodies, including the gut. Both gut microbiota and the neuropeptides oxytocin (OXT) and vasopressin (AVP) are implicated in anxiety-related and social behavior. Our lab has previously found that AVP-immunoreactivity is increased in suprachiasmatic nucleus (SCN) projection sites in juvenile mice raised under germ-free (GF) conditions, with those effects persisting into adulthood in females only. Here, we tested whether OXT immunoreactivity was similarly affected in juveniles, and if recolonization of GF mice at weaning with a complex microbial community from conventionally housed (CC) mice may reverse these effects. We compared OXT immunoreactivity in juvenile (postnatal day 21: P21) and adult (P63) male and female GF or CC Swiss-Webster mice, or born GF and re-colonized with microbiota at 3 weeks of age (RE). Similar to AVP-immunoreactivity, juvenile GF male and female mice show increased OXT-immunoreactivity in the paraventricular nucleus of the hypothalamus and supraoptic nucleus, whereas there is no difference in the bed nucleus of the stria terminalis or the anterior hypothalamus. These results suggest that gut microbiota affect AVP neuropeptide systems in an age-dependent manner and OXT, at least in juveniles, and raises the possibility that AVP and OXT contribute to the gut-brain axis regulation of anxiety-related and social behaviors.

Disclosures: **N.V. Peters:** None. **M.J. Paul:** None. **B. Chassaing:** None. **J. Dunn:** None. **A.T. Gewirtz:** None. **G.J. De Vries:** None.

Poster

445. Neuroimmunology: Regulating Systems

Location: Halls B-H

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Program#/Poster#: 445.08/CCC7

Topic: F.05. Neuroimmunology

Support: NS 085155

Title: Interleukin 18 reduces food intake modulating excitatory synaptic transmission in bed nucleus of the stria terminalis

Authors: *F. BERTON[1], W. FRANCESCONI[1], M. SANCHEZ-ALVAREZ[2], S. ALBONI[4], C. BENATTI[4], S. MORI[1], W. NGUYEN[1], E. ZORRILLA[3], G. MORONCINI[5], F. TASCEDDA[4], B. CONTI[1];
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Abstract: The loss of appetite during sickness is a common and often debilitating phenomenon. Although pro-inflammatory cytokines are recognized as mediators of these effects, their mechanism and sites of action remain poorly understood. Here we show that interleukin 18, previously shown to have anorexigenic effects, acts on neurons of the Bed Nucleus of the Stria Terminalis to reduce food intake via the interleukin 18 receptors. Different cell types in the periphery produce Interleukin 18 while centrally it is present in microglia, ependymal cells and neurons of the medial habenula. The two subunits of the interleukin 18 receptor, - α and - β, are densely expressed in the anterodorsal division of the Bed Nucleus of the Stria Terminalis. We demonstrated that local injection of recombinant interleukin 18, 50 ng/ml, in the anterodorsal division of the Bed Nucleus of the Stria Terminalis, significantly reduced both c-fos activation and food intake for at least 6 hours. Electrophysiological experiments carried out in brain slices containing the Bed Nucleus of the Stria Terminalis demonstrated that the aforementioned dose of interleukin 18 strongly reduced the excitatory input on specific subtype of the Bed Nucleus of the Stria Terminalis type III neurons through a presynaptic mechanism. Interleukin 18 was ineffective in brain slices containing the Bed Nucleus of the Stria Terminalis from interleukin 18 KO mice. Interestingly, type III neurons sensitive to interleukin 18 were located in a restricted area of the Bed Nucleus of the Stria Terminalis defined as the juxtacapsular Bed Nucleus of the Stria Terminalis, which contains neurons projecting to the Lateral Hypothalamus. These results identify a site and a mode of action for interleukin 18 that indicates possible targets for the treatment of cachexia or other eating disorders.

Disclosures: **F. Berton:** None. **W. Francesconi:** None. **M. Sanchez-Alvarez:** None. **S. Alboni:** None. **C. Benatti:** None. **S. Mori:** None. **W. Nguyen:** None. **E. Zorrilla:** None. **G. Moroncini:** None. **F. Tascedda:** None. **B. Conti:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: Swedish Rheumatism Council

Wallenberg Foundation

Title: Vagus nerve stimulation decreases activation of select $CD4^+$ T cell populations and NK cells in mice

Authors: ***J. ESTELIUS**[1,2], K. CHEMIN[1,2], E. LE MAÎTRE[1,2], J. LAMPA[1,2];
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Abstract: Activation of the cholinergic anti-inflammatory pathway (CAP), the efferent branch of the inflammatory reflex, through electrical vagus nerve stimulation (VNS) is known to decrease an inflammatory response via $ChAT^+$ T-cells in the spleen. However, the full extent of the relationship between VNS and the immune system requires further investigation. To elucidate changes in the immune response to endotoxaemia induced by stimulation of the vagus nerve, male C57BL/6 mice underwent VNS or sham surgery after i.p. injection of lipopolysaccharides (LPS). This was followed by organ collection and cell preparation 6h post-surgery. Flow cytometric analysis revealed that VNS significantly reduced the expression of the early activation marker CD69 on $CD4^+$ T-cells in spleen and blood ($t(4)=-3.3$, $p<0.05$ and $t(4)=-4.0$, $p<0.05$ respectively). Further analysis of the $CD4^+$ T-cell compartment demonstrated significant CD69 reduction in both naïve ($CD44^{low}$, $CD62L^{high}$) ($t(4)=-3.3$, $p<0.05$, and $t(4)=-4.2$, $p<0.05$, spleen and blood respectively) and memory ($CD44^{high}$, $CD62L^{low}$) ($t(4)=-3.7$, $p<0.05$, spleen) T-cells after VNS. Interestingly, $CD335^+$ NK cells also showed a significant reduction in CD69 up-regulation with VNS ($t(5)=-3.4$, $p<0.05$), without effects on cell numbers. Conversely, $FoxP3^+$ regulatory $CD4^+$ T-cells as well as B-cells remained unaffected by VNS regarding both cell numbers and CD69 expression. Collectively, our data indicate that immune suppression through VNS selectively affects early endotoxaemia-induced activation in both spleen and blood, as measured by inhibition of CD69 up-regulation, in $CD4^+$T-cells of both naïve and memory

phenotype, as well as in NK-cells. No effect was observed in either regulatory $CD4^+$ T-cells or B-cells. These findings are in line with general immunosuppressive effects on T-cell subsets. Moreover, we detected a previously unknown VNS mediated suppression of NK-cell activation, which will increase our understanding of neuro-immune regulation pathways and may contribute to the development of novel immunomodulatory therapeutic strategies in chronic inflammatory diseases.

Disclosures: J. Estelius: None. **K. Chemin:** None. **E. Le Maître:** None. **J. Lampa:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: NIH Grant R15NS083532

Title: Delayed contribution of hematopoietically-derived central nervous system macrophages in mixed chimeric mice utilizing the myeloablative compound Busulfan

Authors: M. I. ROCHA, K. B. PRUNER, J. M. KURTZ, *T. D. WILLIAMS;
Biol., Emmanuel Col., Boston, MA

Abstract: Several lines of evidence suggest there is minimal recruitment of peripheral monocytes to central nervous system (CNS) macrophage population in the absence of disease. Therefore, CNS inflammation may actively recruit peripheral immune cells in the response to disease. However, turnover of hematopoietically-derived immune cell precursors may also influence contribution to the CNS over time in non-disease states. To study this contribution, we established mixed syngeneic chimeric mice utilizing the myeloablative drug Busulfan followed by a hematopoietic stem cell transplant (HSCT) of GFP^+ donor cells into C57BL/6 recipient mice (n=30). Recipient mice were administered Busulfan (30mg/kg body weight) 7, 5, and 3 days prior to HSCT, followed by transplantation of $20*10^6$ bone marrow cells from C57BL/6-Tg(UBC-GFP) donor mice. Blood chimerism levels were analyzed through flow cytometry and yielded greater than 90% donor-derived monocytes by 1 week post-HSCT. Beginning at 8 weeks post-HSCT, approximately 2-3% of CNS macrophages were $CD11b^+$ $CD45^{mid}$ GFP^+. Supporting immunofluorescence evidence of CNS donor-derived macrophages was observed by Iba-1^+GFP^+ non-ramified cells in the choroid plexus as well as Iba-1^+GFP^+ ramified cells in the brain parenchyma. To further study how increased HSC proliferation affects donor cell contribution to CNS macrophage populations, chimeric mice (n=5; 45 weeks post-HSCT) were treated with an

additional Busulfan administration (25mg/kg body weight on two separate days). Following this partial myeloblation stress, we observed consistent high levels (>90%) of peripheral blood monocyte chimerism and no change in $CD11b^{+}CD45^{mid}GFP^{+}$ CNS cells at 8 weeks, however at 13 weeks (n=2), an increase to ~6% $CD11b^{+}CD45^{mid}GFP^{+}$ CNS cells was observed. Taken together, these data suggest the establishment of peripheral chimerism needs to occur prior to any significant contribution of hematopoietically-derived precursor cells to the CNS macrophage population.

Disclosures: **M.I. Rocha:** None. **K.B. Pruner:** None. **J.M. Kurtz:** None. **T.D. Williams:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: NIH R01HL082517

Title: Molecular mechanisms of csf hypersecretion in intraventricular hemorrhage-associated hydrocephalus.

Authors: *J. K. KARIMY[1,2], D. B. KURLAND[1], B. CARUSILLO[1], J. ZHANG[3], V. GERZANICH[1], J. SIMARD[1], K. T. KAHLE[2];
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Abstract: Intraventricular hemorrhage (IVH), is the accumulation of blood within the ventricles, and is associated with high morbidity and mortality via IVH-associated hydrocephalus (IVHH). IVHH distorts the surrounding brain tissue, resulting in cognitive and motor impairments, or death. IVHH is attributed to impaired CSF reabsorption at the arachnoid granulations; however, the contribution of CSF hypersecretion is unknown. NKCC1 is regulated by SPAK via phosphorylation, and is critical to the formation of CSF. A novel method to measure the rate of CSF secretion was used to study the role of the SPAK-NKCC1 pathway in IVHH. IVH resulted in ventriculomegaly, due to a 3-fold increase in CSF secretion, determined to be due to SPAK-mediated phosphorylation of NKCC1. Methods to inhibit phosphorylation of NKCC1 significantly attenuated the hypersecretion of CSF and ventriculomegaly associated with IVHH.

These results provide evidence that the SPAK-NKCC1 pathway drives the pathogenesis of IVHH and is a potential therapeutic target.

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Poster

445. Neuroimmunology: Regulating Systems

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Title: Immune regulation and mesenchymal stromal cell-produced pain relief: 2. role of NFkappaB signaling and regulatory T cells

Authors: *W. GUO[1], S. IMAI[1,2], S. ZOU[1], F. WEI[1], R. DUBNER[1], K. REN[1];
[1]Dept. of NPS, Dent. School, & Prog. in Neurosci, Univ. of Maryland, Baltimore, MD; [2]Dept. of Clin. Pharmacol. & Therapeut., Kyoto Univ. Hosp., Kyoto, Japan

Abstract: Bone marrow stromal cells (BMSCs), a major type of mesenchymal stromal cells, produce long-lasting attenuation of pain hypersensitivity after tissue or nerve injury. This effect involves BMSC's ability to induce an anti-inflammatory phenotype, represented by increased anti-inflammatory cytokine production and alternatively activated monocytes/macrophages population. We further studied the effect of BMSC on NF-κB and the regulatory T cells (Tregs), the two upstream events that are important for cytokine regulation and monocyte polarization. In Sprague Dawley rats with a ligation injury of the masseter tendon (TL), BMSCs (1.5M/rat) were infused i.v. at 1 w post-TL. At 1 w, but not 8 w after BMSC infusion, western blot and immunostaining showed that p65 of NF-κB was significantly increased in the rostral ventromedial medulla (RVM), a key structure in endogenous pain modulation that has been shown to be involved in BMSC-produced pain relief. P65 exhibited predominant neuronal localization in the RVM with scattered distribution in glial cells, as shown by double immunofluorescence with NeuN, GFAP and CD11b. At 7 d post-BMSC infusion when there was

clear antihyperalgesia, BAY 11-7082 (0.5 pmol/0.5μl), an inhibitor of NF-κB signaling, was injected into the RVM. Compared to vehicle-injected rats, BAY 11-7082 attenuated BMSC-produced antihyperalgesia, as indicated by a significant increase in mechanical sensitivity to von Frey filament probing. These results suggest that NF-κB transcriptional activation contributes to BMSC-produced pain relief. The $CD4^+CD25^+Foxp3^+$regulatory T cells (Tregs) have been shown to induce alternative activation of monocytes/macrophages and TGF-β induces $CD4^+$ $CD25^+Foxp3^+$Tregs from native $CD4^+$ T cells. The NF-κB activity is likely down-stream to these signaling events. Our RT-PCR results showed that TGF-β expression in primary BMSCs that produced pain relief was 2-fold higher than 20-passage BMSCs that did not produce pain relief in rats, consistent with a role of TGF-β in inducing Tregs for BMSC-induced antihyperalgesia. Flow cytometry was performed on peripheral blood cells after removing red blood cells from C57B mice with triple labeling and appropriate isotope controls. Compared to naïve mice, the frequency of $CD4^+CD25^+Foxp3^+$Tregs among the $CD4^+$ population was decreased in mice with injury of the infraorbital nerve, but increased to above the naïve level in the nerve-injured mice at 8 w after receiving BMSC. Taken together, these results suggest a scenario that BMSC-secreted mediators induce Tregs, followed by subsequent activation of anti-inflammatory phenotype in the host, leading to long lasting pain relief.

Disclosures: **W. Guo:** None. **S. Imai:** None. **S. Zou:** None. **F. Wei:** None. **R. Dubner:** None. **K. Ren:** None.

Poster

445. Neuroimmunology: Regulating Systems

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5T32HL007633

Title: Airway nociceptors: Allergen sensing and reflex responses

Authors: ***S. TALBOT**[1], S. L. FOSTER[1], M. A. PASCAL[1], R. B. CHANG[2], A. PARRIN[1], B. DOYLE[1], N. Y. LAI[1], C. LAEDERMANN[1], R.-E. E. ABDULNOUR[4], A. LATREMOLIERE[1], L. BROWNE[1], S. D. LIBERLES[2], B. P. BEAN[3], B. D. LEVY[4], C. J. WOOLF[1];
[1]F.M Kirby Neurobio. center, Children's Hosp. Boston., Boston, MA; [2]Dept. of Cell Biol., [3]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [4]Pulmonary and Critical Care Med. Division, Dept. of Intrnl. Med., Brigham and Women's Hosp., Boston, MA

Abstract: In vertebrates, the immune and nervous systems have co-evolved complex and complementary mechanisms to detect danger and protect tissue from damage. This functional integration includes bidirectional communication between the two systems that can become maladaptive in disease situations. We have found that lung nociceptor neurons mediate a feedforward inflammatory loop during allergic inflammation by both sensing and responding to type 2 inflammatory cytokines and by fueling immune cells influx through local neuropeptide release (Talbot et al, Neuron 2015). The cellular mechanisms that engage airway nociceptors to initiate this cascade remain, however to be identified. Calcium imaging and electrophysiological recording reveal that nodose ganglion neurons from allergen (ovalbumin) sensitized mice respond with activation to the allergen when it is complexed with immunoglobulin subtype E (IgE), a response absent in FcεR1α knockout mice. Stimulated nociceptors in turn locally release Substance P (SP) which act on lung goblet cells to trigger mucus secretion, mucin imbalance (Muc5AC/Muc5B) and accumulation. During allergic inflammation, the ablation of TRPV1+ sensory neurons or silencing with QX-314, a charged sodium channel blocker that enters via large pore ion channels to specifically block nociceptors, reverse these effects. Optogenetic activation of $Tac1^{+}$ vagal sensory neurons also produce inflammation leading to mucus secretion and mucin imbalance. Our results indicate that airway nociceptors from sensitized animals sense allergens, which in turn release neuropeptides that induce mucus secretion. Silencing nociceptor neurons with QX-314 resolves neurogenic inflammation and mucus metaplasia and targeting the nervous system represents therefore a new therapeutic strategy for airway allergic diseases.

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Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: DARPA W911NF-09-1-0125

Title: Murine cervical vagus nerve activity: methodology of recording and analysis

Authors: *H. A. SILVERMAN[1,2], B. E. STEINBERG[3], T. TSAAVA[1], A. STIEGLER[4], E. A. BATTINELLI[1,2], J. NEWMAN[1], A. CARAVACA[5], S. ROBBIATI[6], P. T. HUERTA[6,2], S. S. CHAVAN[1], K. J. TRACEY[1,2];
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Abstract: Sensory vagus nerve fibers transmit action potentials to the central nervous system in response to changes in the body's metabolic and physiological status. Here we developed a method for recording compound action potentials in the cervical vagus nerve of adult mice. For vagus nerve recordings, animals were induced (isoflurane at 2%) and maintained (isoflurane at 1.75%) in the supine position. The left cervical branch of the vagus nerve isolated from the accompanying carotid bundle, and was placed over three custom-built silver hook electrodes (n=72) or in a commercially available bipolar sling cuff electrode (n=25) with 150 μm inner diameter (CorTec, Germany). Electrophysiological signals were digitized (sampling rate, 32 kHz) through a data acquisition system (Digital Lynx 4SX, Cheetah v5 software, Neuralynx, Bozeman, MT) and referenced to an animal ground electrode placed between the skin and right salivary gland. Ten minutes of baseline vagus nerve activity was recorded (hook electrode: 4.19 spikes/s ± SEM 0.87 spikes/s, cuff electrode: 4.86 spikes/s ± SEM 2.65 spikes/s). Recordings with a cuff electrode demonstrated a significantly lower background electrical activity as compared to the hook electrode ($p<.001$). Next, we determined whether there are any strain-specific differences in the baseline vagus nerve activity by comparing vagus nerve activity between adult Balb/c and B6.129s mice. No significant difference was observed in the spike rate over time or the total power between strains. To assess whether nutritional status affects the baseline vagus nerve activity animals were fasted prior to the recording. Non-fasted mice demonstrated a significantly higher amount of total spikes over the 10-minute period ($p<0.5$) as compared to the fasted group. Changes in the vagus nerve activity were then recorded in response to the administration of an inflammatory mediator. Following acquisition of baseline activity, animals received increasing amounts of bacterial lipopolysaccharide (LPS), and compound action potentials were recorded in the vagus nerve. Administration of LPS induced a significant increase in the vagus nerve activity. These studies report a novel method for recording changes in the cervical vagus nerve activity in mice.

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Poster

445. Neuroimmunology: Regulating Systems

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Program#/Poster#: 445.15/CCC14

Topic: F.05. Neuroimmunology

Title: Investigating the effects of inflammation and minocycline on central glutamate receptors and the metabolome

Authors: *S. CHAN[1], F. PROBERT[2], D. C. ANTHONY[2], P. W. J. BURNET[3];
[2]Pharmacol., [3]Psychiatry, [1]Univ. of Oxford, Oxford, United Kingdom

Abstract: Background and Objective: Given the strong association between pro-inflammatory cytokines and psychiatric disorders, drugs with anti-inflammatory properties, such as minocycline, have gained interest as possible therapeutic agents. However, the mechanisms underlying their psychotropic properties are unclear. At the molecular level, studies linking the pro-inflammatory cytokine IL-1β to NMDA receptors suggest that central glutamate neurotransmission may be involved. We therefore examined the effect of minocycline and inflammation on glutamate receptor gene expression in the mouse brain. In addition, we used metabolomics to screen for other small molecules that may be affected by this drug.
Methods: Minocycline or saline was administered to male CD1 mice for seven days, followed by 24 hour-treatment with the inflammatory agent LPS, or saline. Region-specific central glutamate receptor gene expression was determined with in-situ hybdridisation. Statistical analyses were conducted with two-way ANOVA to determine the main effects of LPS and minocycline, as well as interactions between minocycline and LPS.
^{1}H-NMR spectroscopy was used to investigate the peripheral and central effects of minocycline on metabolites. Mice were treated with an acute dose of saline or minocycline for two hours before tissue and plasma harvesting. Samples were ran on a 700MHz system and processed to obtain spectra.
Results: LPS administration decreased NR2A (-33.44%, $p<0.01$) and NR2B (-13.76%, $p<0.05$) mRNA in the CA1 region of the hippocampus. This effect was modulated by pre-treatment with minocycline, with a significant minocycline x LPS interaction for NR2A ($F_{1,15} = 16.411$, $p<0.01$) and NR2B ($F_{1,15} = 18.319$, $p<0.01$) mRNA.
Preliminary metabolomic analyses have revealed significant differences between control and treatment groups by PCA and PLS-DA (predictive q^2 value>0.4) in plasma samples. Common brain metabolites have been identified in the brain extracts, and samples are currently being analysed. A second study involving 7-day pre-treatment with minocycline followed by administration with LPS will be conducted. We predict that clear metabolic profiles will be seen for each group, with the minocycline/LPS-treated group possibly modulating changes seen in the saline/LPS-treated group.

Conclusion: As dysregulation of glutamate receptors has been linked to the pathology of psychiatric disorders, it is possible that minocycline functions by modulating NMDA receptors. However, as minocycline has been shown to affect many targets, looking at changes in the metabolome would enable us to identify other non-immune pathways that may be involved.

Disclosures: S. Chan: None. **F. Probert:** None. **D.C. Anthony:** None. **P.W.J. Burnet:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Title: Immune regulation and mesenchymal stromal cell-produced pain relief: 1. promotion of anti-inflammatory phenotype

Authors: S. IMAI[1,2], W. GUO[1], S. ZOU[1], F. WEI[1], R. DUBNER[1], *K. REN[1];
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Abstract: Recent studies have pointed to a promising pain-relieving effect of mesenchymal stromal cells in animal models of persistent pain. A single transfusion of autologous bone marrow stromal cells (BMSCs), a major type of mesenchymal stromal cells, attenuates behavioral pain hypersensitivity after tissue or nerve injury and the pain attenuation lasts for months. BMSCs are known to alter inflammatory responses to injury. To understand cellular mechanisms underlying BMSC-produced pain relief, we studied the effect of BMSCs on pro- and anti-inflammatory cytokines in a rat model of myogenic orofacial pain. We focused on rostral ventromedial medulla (RVM), a key structure in endogenous pain modulation that has been shown to be involved in BMSC-produced pain relief. Injury was induced by ligation of one masseter muscle tendon (TL) of the rat via an intraoral approach. We have shown that TL induces persistent pain that can be attenuated by BMSCs. BMSCs were collected from the rat and cultured. At 1 w after TL, BMSCs (1.5 M cells/rat, i.v.) were infused and RVM tissues were

collected. Western blot and immunostaining showed that BMSCs reversed TL-induced upregulation of IL-1β expression in the RVM at 8 w, but not at 1 w, after BMSC infusion. Interestingly, the suppressor of cytokine signaling 3 proteins, a feedback inhibitor of signaling pathways related to cytokine transcription, was significantly upregulated at the 8 w time point. On the contrary, the levels of an anti-inflammatory cytokine IL-10 were increased in the RVM at 1 and 8 w after BMSC infusion. Double immunofluorescence showed that RVM IL-10 colocalized with neuronal marker NeuN and astrocytic marker GFAP. Since resident microglia may be modulated to express alternatively activated anti-inflammatory M2 phenotype after BMSC treatment and IL-10 is an activator of M2 phenotype, we further examined M2 marker in the RVM. RT-PCR showed that CD206 (mannose receptor), a marker of M2 macrophages/microglia, was upregulated in the RVM of TL rats after BMSC treatment. Immunostaining showed that CD206 selectively co-localized with CD11b, a microglial marker, in the RVM. Consistently, we found increased gene expression of M2 markers *CD206*, *CD163* and *Irf4* (Interferon regulatory factor 4) and decreased gene expression of proinflammatory M1-related genes *Irf5* and *Cxcl10* in peripheral blood monocytes derived from BMSC-treated TL rats. Together with our previous behavioral observations, these results suggest that BMSCs facilitate the acquisition of the M2 antiinflammatory phenotype in the RVM as well as induce M2 polarization in periphery in injured animals. These effects of BMSCs should contribute to their pain-relieving effect.

Disclosures: S. Imai: None. **W. Guo:** None. **S. Zou:** None. **F. Wei:** None. **R. Dubner:** None. **K. Ren:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

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Title: Neuronal regulatory RNAs and neuropsychiatric lupus

Authors: *A. IACOANGELI[1], I. MUSLIMOV[1], H. TIEDGE[1,2];
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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune disease affecting an estimated 2 million US Americans. Neuropsychiatric SLE (NP-SLE), a common occurrence in lupus patients, is characterized by neurological manifestations including psychosis and seizures. Regulatory Brain Cytoplasmic (BC) RNAs are translational regulators at the synapse. Mice lacking BC1 RNA are susceptible to hyperexcitability and epileptogenesis. BC RNAs are delivered to synapto-dendritic subdomains by heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2). hnRNP A2 recognizes a dendritic transport element (DTE), a GA motif located in the 5' region of BC RNAs that is required for dendritic targeting. Sera of lupus patients have been reported reactive to double stranded DNA and double-stranded RNA. We found that 77% of screened SLE patients had a strong immune response to BC RNAs. Using electrophoretic mobility shift assays (EMSAs), we observed that IgGs from SLE sera formed complexes with BC200 RNA and BC1 RNA. The majority of lupus patients showing strong immune reactivity to BC RNAs were NP-SLE patients with neurological manifestations. Complex formation was not detected with IgGs from patients with other systemic or localized autoimmune diseases (e.g. rheumatoid arthritis or multiple sclerosis). Autoantibodies against BC RNAs (anti-BC abs) bind to the DTE that is recognized by hnRNP A2. Competition assays revealed that hnRNP A2 and anti-BC abs interact with DTEs of BC RNAs in a competitive manner. Hypothesizing that anti-BC IgG competition with hnRNP A2 compromises dendritic BC RNA transport, we co-microinjected sympathetic neurons in culture with radiolabeled BC RNAs and anti-BC IgGs. We observed that dendritic BC RNAs transport was inhibited in the presence of anti-BC abs. Delivery of BC RNAs to dendritic subdomains was not affected when IgGs from non-SLE subjects were used in the co-injection experiments.
The data indicate that anti-BC IgGs, competing with hnRNP A2, disrupt delivery of regulatory BC RNAs to dendrites. Such competition may impact BC RNA functional activity at the synapse.

Disclosures: **A. Iacoangeli:** None. **I. Muslimov:** None. **H. Tiedge:** None.

Poster

445. Neuroimmunology: Regulating Systems

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NARSAD Young Investigator Grant

Title: Age-associated circadian dysregulation in rats may sensitize neuroinflammatory responses

Authors: *L. K. FONKEN, M. M. KITT, A. D. GAUDET, R. M. BARRIENTOS, L. R. WATKINS, S. F. MAIER;
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Abstract: Aged animals exhibit diminished molecular and behavioral circadian rhythms. Both aging and circadian disruption sensitize neuroinflammatory processes. Thus, here we explored whether aging is associated with disrupted diurnal rhythms in neuroinflammatory processes. These experiments were conduced with F344XBN rats and specifically focused on the hippocampus as potentiation of the inflammatory response is particularly robust in the aged hippocampus. Here we show that hippocampal tissue isolated from adult (4 mos.) rats showed increased markers of microglia activation (Iba1 and CD68 mRNA expression) during the middle of the light phase. In contrast, there were no diurnal differences in markers of microglial activation in aged (25 mos.) rats; aged rats exhibited constitutively elevated Iba1 and CD68 mRNA expression. Interestingly, there were no diurnal differences in GFAP expression in young or aged rats. Consistent with previous findings, there was an overall elevation in GFAP in aged rats. Next, we explored whether aging is associated with disrupted diurnal rhythms in microglia isolated from the hippocampus. Hippocampal microglia were isolated from adult and aged rats every 6h. While microglia from young rats rhythmically expressed core circadian clock genes, microglia from aged rats had atypical non-rhythmic expression of several clock genes, including *Per1* and *Per2*. Furthermore, unstimulated (non-LPS treated) microglia from young rats exhibited robust rhythms of *TNFα* and *IL1β* mRNA, with peak expression during the middle of the light phase. In contrast, unstimulated microglia isolated from aged rats had tonically elevated inflammatory cytokines that were not regulated by time-of-day. Similarly, diurnal differences in responsiveness to both *ex vivo* and *in vivo* inflammatory challenges were abolished in aged rats. Corticosterone treatment induced Per1 expression in aged and young microglia; however, glucocorticoid rhythms are suppressed in the aged hippocampus. This suggests that changes in entrainment signals may be disrupted with aging. Overall, here we show that intrinsic microglial rhythms in clock and inflammatory genes are dysregulated with aging and may contribute to pathological neuroinflammatory responses.

Disclosures: L.K. Fonken: None. **M.M. Kitt:** None. **A.D. Gaudet:** None. **R.M. Barrientos:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

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Topic: F.05. Neuroimmunology

Support: R01AA12882

Title: Involvement of hypothalamic CCL2/CCR2 chemokine system in the stimulatory effects of maternal exposure to low-dose ethanol on embryonic development of orexigenic peptide neurons in rats

Authors: *S. F. LEIBOWITZ, G.-Q. CHANG, O. KARATAYEV;
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Abstract: Background: Clinical and animal studies show that maternal consumption of alcohol during pregnancy increases alcohol drinking in the offspring and also stimulates neurogenesis and density of hypothalamic neurons that express orexigenic peptides known to increase alcohol intake. With studies showing ethanol to activate the peripheral immune system and increase circulating levels of the chemokine CCL2, we recently investigated in Sprague-Dawley rats (Chang et al., 2015) the effect of ethanol on neuroimmune function in the hypothalamus. We found that maternal oral administration of ethanol (1 or 3 g/kg/day, i.g.) from embryonic day 10 (E10) to E15, compared to control isocaloric solution of maltose-dextrin, markedly increases the birth and density of neurons in the perifornical lateral hypothalamus (PFLH) that express melanin-concentrating hormone (MCH) and co-label the chemokine receptor CCR2, while also stimulating the drinking of ethanol in pubertal offspring. These findings agree with other evidence showing alcohol intake to be reduced by knockout of CCR2 and increased by PFLH injection of MCH. **Methods:** Quantitative real-time PCR and immunofluorescence histochemistry were used to further examine the relationship between the CCL2/CCR2 system and development of MCH neurons in PFLH. **Results:** Using confocal microscopy, we found that, as compared to only 30% in control rats, almost 100% of MCH neurons in PFLH co-express CCR2 in pre-adolescent offspring prenatally exposed to ethanol (1 g/kg/day, E10-E15). We also showed that peripheral administration to dams of CCL2 itself (2-4 μg/kg, s.c.) increases maternal circulating CCL2 (+45%, $p<0.05$) to the same level (530-553 pg/ml) induced by oral administration of ethanol (1 g/kg, i.g.). We then tested whether CCL2 injection has similar effects to ethanol and possibly mediates ethanol's stimulatory effects on neurons that co-label CCR2 and MCH. We found that, like ethanol, daily injections in the dam (E10-E15) of CCL2 (2 μg/kg/day), compared to its purified IgG vehicle, significantly increase in offspring ($p<0.01$) mRNA levels of MCH and density of CCR2-labeled cells in PFLH. Also, in dams administered oral ethanol (1 g/kg/day), we found daily injections (E10-E15) of the CCR2 antagonist, INCB3344 (1 mg/kg/d, i.v.), compared to its PBS vehicle to have opposite effects to CCL2, causing a reduction in both MCH mRNA and density of CCR2-labeled cells ($p<0.01$). **Conclusions:** These findings provide evidence for a stimulatory effect in offspring of maternal CCL2, like ethanol, on the density and expression of orexigenic peptide neurons in PFLH that co-express CCR2 and a role for CCR2 in mediating ethanol's effects.

Disclosures: S.F. Leibowitz: None. **G. Chang:** None. **O. Karatayev:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: Swedish research council

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Title: Nicotine mediated immunosuppression is compromised in lipopolysaccharide activated splenocytes from microsomal prostaglandin E synthase–1 knock–out mice

Authors: *P. REVATHIKUMAR, U. KARMAKAR, E. LE MAÎTRE, M. KOROTKOVA, P. JAKOBSSON, J. LAMPA;
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Abstract: Vagus nerve stimulation (VNS) is effective in controlling inflammation. Interestingly, we showed recently that the VNS mediated immunosuppression is impaired in mice lacking microsomal prostaglandin E synthase-1 expression (mPGES-1), a key enzyme responsible for prostaglandin (PG) E_2 synthesis. In addition, mPGES-1 KO mice display defective splenic acetylcholine release in response to VNS. Here we aim to investigate the cholinergic system of VNS treated mPGES-1 KO mice.

Methods

Animals: All experiments were performed according to local Ethical Committee guidelines. Both WT and mPGES-1 KO mice were injected with lipopolysaccharide (LPS) (2 mg/kg; i.p). After VN isolation, it was either electrically stimulated for 5 minutes (VNS) or left undisturbed (Sham). After 30 minutes, spleens were collected for NE and choline measurement.

***In vitro* studies**

(i) Splenocytes were seeded in chamber slides, treated with LPS (10ng/ml) for 20 hours and stained for choline acetyltransferase (ChAT) expression using immunofluorescence.

(ii) Splenocytes were pretreated with nicotine at 100μM for 30 minutes, later stimulated with LPS (10ng/ml) for 3, 6 and 20 hours respectively and supernatants were collected for TNF α measurement.

Results

(i) No difference was detected concerning splenic NE concentration in mPGES-1 KO mice vs WT following VNS (10.4±2.2 vs 9.6±3.4 pg/ml). However, VNS tended to increase the choline content in WT spleen by 6% (n=5) whereas no such increase was observed in mPGES-1 KO mice.

(ii) LPS treatment induced strong ChAT expression in both WT and mPGES-1 KO splenocytes. However, the % of cells positive for ChAT expression was more pronounced in WT compared to

mPGES-1 KO mice. (81.2% WT vs 55.8% mPGES-1 KO).
(iii) Nicotine treatment (3 hours only) strongly inhibited LPS induced TNF α production (25±8.7 % inhibition, $p<0.05$, $n=4$) in WT. On the contrary, nicotine failed to exhibit similar effects on activated mPGES-1 KO splenocytes.

Conclusion

VNS induces splenic NE release in both WT and mPGES-1 KO to similar extent. However, subsequent increase in splenic choline production seen in WT is absent in mPGES-1 KO. In addition, LPS treatment tends to increase ChAT expression more strongly in WT compared to mPGES-1KO. Importantly, lack of mPGES-1 expression in splenocytes reversed nicotine's immunomodulating effects on TNF α release. In summary, defective cholinergic synthesis and inability to respond to cholinergic stimulation are likely to be key aspects explaining the failure of VNS to reduce LPS induced inflammation in mPGES-1 KO mice. Our results shed light on the possible link between cholinergic and PG system that may be of clinical significance in VNS treatment.

Disclosures: **P. Revathikumar:** None. **U. Karmakar:** None. **E. Le Maître:** None. **M. Korotkova:** None. **P. Jakobsson:** None. **J. Lampa:** None.

Poster

445. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

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Title: Estradiol enhances microglial reactivity in the ventromedial hypothalamus of pubertal female mice

Authors: *A. VELEZ[1], S. FOUNTAIN[2], J. D. BLAUSTEIN[1];
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Abstract: Pubertal development is a period of drastic transformation in central nervous system. Brain regions regulating reproduction undergo significant structural changes by mechanisms that include neurogenesis, apoptosis and synaptic pruning. Microglia, the resident immune cells of

the brain, are critical for appropriate maturation of the brain. At basal conditions they nurse synaptic connections, survey the environment and secrete cytokines. However, after an immune challenge microglia undergo a drastic change in morphology to support their macrophage function clearing cellular debris and phagocytizing pathogens, increasing cytokine production and becoming antigen-presenting cells. One of the hallmarks of pubertal development is the maturation of the reproductive axis, which results in increased secretion of ovarian hormones, including estradiol. In two experiments, we tested the hypothesis that ovarian secretions influence the response of microglia to the immune stressor, lipopolysaccharide (LPS), during pubertal development. In the first experiment, female mice were ovariectomized and received an oil or estradiol capsule 7 days prior to LPS or saline injection on P42 (during pubertal development). In the second experiment, gonadally-intact females received an aromatase inhibitor or vehicle injection for 7 consecutive days prior to injection of LPS or saline on P42. Animals were euthanized 24 hours post-injection and brains were collected. Microglia were identified by immunohistochemistry using an antibody against ionized calcium binding adapter molecule-1 (Iba1), which is expressed in microglia. We measured mean Iba-1 immunoreactivity to determine relative changes in the mean area covered by microglia, which serves as a proxy of microglial reactivity. Estradiol treatment resulted in a greater increase in microglial immureactivity in the ventromedial hypothalamus (VMH), but not the arcuate nucleus (ARC). In addition, blocking estradiol synthesis decreased microglial immunoreactivity in the VMH, but not the ARC. Lastly, as expected, LPS treatment increased microglial density in the VMH and the ARC compared to saline controls. Overall, these results suggest that estradiol modulates microglial reactivity in a brain-region dependent manner.

Disclosures: **A. Velez:** None. **S. Fountain:** None. **J.D. Blaustein:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

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The Michael J Fox Foundation

Title: Interleukin-13 receptor alpha 1 contributes to loss of dopaminergic neurons in a model of chronic stress

Authors: *S. MORI[1], S. SUGAMA[2], W. NGUYEN[1], G. MORONCINI[3], Y. KAKINUMA[2], P. MAHER[4], B. CONTI[1];
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Abstract: Parkinson's disease is the second most common progressive neurodegenerative disease affecting more than 2 million people in the United States and is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta. The vast majority of Parkinson's disease is idiopathic and believed to be the result of an interaction between genes and the environment. Exposure to chronic stress has proven to be an environmental factor that can damage dopaminergic neurons but the mechanisms by which this occurs remain to be determined. Here we tested the hypothesis that interleukin-13 receptor alpha 1, the putative PARK 12 expressed in dopaminergic neurons, can contribute to the vulnerability of these cells during stress in the mouse. We found that acute restraint stress stimulates the synthesis of interleukin-13 in the substantia nigra pars compacta where this cytokine was localized in microglia as well as in neurons. We also measured and compared the effects of chronic restraint stress on nitrotyrosine and on the number of dopaminergic neurons in the substantia nigra pars compacta of mice null for interleukin-13 receptor alpha 1 and their wild type littermates. We found that the loss of dopaminergic neurons during chronic stress was delayed in *Il13ra1*$^{-/Y}$ mice indicating that interleukin-13 receptor alpha 1 is one factor that contributes to the stress-mediated damage of dopaminergic neurons.

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Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Title: Altered experience-dependent interleukin-1 beta signaling in juvenile rats exposed to ethanol as neonates

Authors: *M. J. GOODFELLOW, D. H. LINDQUIST;
Psychology, Ohio State Univ. Dept. of Psychology, Columbus, OH

Abstract: Fetal alcohol spectrum disorders (FASD) afflict up to 5% of American school age children (May et al., 2009), many of whom experience pervasive cognitive deficits, including impaired long-term memory (Jacobson et al., 2011). Ethanol-induced neuroinflammation is a major factor in FASD etiology (Alfonso-Loeches and Guerri, 2011)—both acute and chronic increases in basal pro-inflammatory cytokine interleukin (IL)-1β expression have been detected in the hippocampus of rodents exposed to ethanol during a human 3rd trimester equivalent period on postnatal day (PD) 10 (Drew et al., 2015) and PD28 (Tiwari and Chopra, 2011). While immune molecules in the healthy brain are important for synaptic plasticity and memory (del Ray et al., 2013), aberrant IL-1β expression can interfere with long-term potentiation (LTP) maintenance and long-term memory consolidation (Rachal Pugh et al., 2001). In addition to inducing changes in basal IL-1β expression (Wang et al., 2013), neonatal neuroinflammation has been shown to exaggerate experience-dependent IL-1β release in the hippocampus of adult rats following fear conditioning, resulting in impaired associative learning and memory (Williamson et al., 2011). It is currently unknown, however, if experience-dependent release is also altered by neonatal ethanol exposure.

In this study, male and female rats are administered ethanol (5 g/kg/day; 5E) via intragastric intubation or sham intubated (SI) across a 3rd trimester equivalent period (PD4-9) and, as juveniles (PD31), submitted to a hippocampus-dependent associative learning task, trace fear conditioning (TFC). Experience-dependent IL-1β mRNA and protein is measured in the dorsal hippocampus of separate rats 2 h following the completion of conditioning; basal IL-1β expression is also measured in experimentally naïve SI and 5E home cage control rats. Consistent with prior studies in adults and adolescents (Goodfellow et al., 2016; Schreiber and Hunt, 2013), preliminary results indicate that juvenile 5E rats freeze less than SI control rats during CS-alone test trials. Current data also suggest that the observed impairments are due, at least in part, to altered experience-dependent release of IL-1β, possibly impeding LTP maintenance and memory consolidation. If correct, then pharmaceutical interventions that modify IL-1β signaling during and/or immediately after TFC may strengthen synaptic plasticity and long-term memory consolidation, resulting in improved TFC test performance in 5E rats. Positive results would also suggest that immunomodulating drugs could be efficacious treatments for cognitive dysfunction in people with FASD.

Disclosures: M.J. Goodfellow: None. **D.H. Lindquist:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Title: Chronic cocaine self-administration alters cognitive flexibility in male HIV transgenic rats

Authors: *S. E. HEMBY[1], S. MCINTOSH[2];
[1]Basic Pharmaceut. Sci., Fred Wilson Sch. of Pharmacy/High Point Univ., High Point, NC;
[2]Basic Pharmaceut. Sci., Fred Wilson Sch. of Pharmacy/High Point Universi, High Point, NC

Abstract: HIV infection and cocaine dependence can both disrupt neural circuitry mediating executive functions involved in decision-making and cognitive flexibility. The objective of the current study was to determine the impact of chronic cocaine intake on reversal learning in the HIV transgenic rat model. Previously, our lab has shown that male HIV transgenic rats are more sensitive to the reinforcing effects of cocaine accompanied by significant changes in the affinity of the dopamine transporter in the striatum. The present study compared the acquisition and performance on an operant reversal-learning task between male HIV-1 transgenic and Fischer F344 rats before and following chronic cocaine self-administration. In addition, in vivo microdialysis was used to compare extracellular dopamine levels in the striatum during a cocaine self-administration session. Performance on reversal learning tasks was assessed prior to the initiation of self-administration procedures and following cocaine self-administration. Rats were trained to intravenously self-administer cocaine (41ug/infusion) under a fixed ratio 3 (FR3) schedule of reinforcement during daily sessions. Sessions consisted of a 3 hr or 40 maximum infusion trial that was preceded by a priming infusion. Once animals reached a total intake criterion of 220mg of cocaine, animals were assessed with reversal learning. After the second assessment of reversal learning, microdialysis probes were inserted through previously implanted guide cannula in the ventromedial striatum. In summary, results demonstrate that HIV-1 Tg rats are capable of learning an operant spatial discrimination task and to reverse the spatial discrimination. After chronic cocaine self-administration, HIV-1 Tg rats showed impairment in the reversal and retention of a previously learned spatial discrimination compared to F344 rats. Basal levels of dopamine detected in microdialysates of the HIV Tg rats (5.2 nM ± 1.2 SEM) were lower compared to the F344 rats (10.2 nM ± 2.1 SEM) [p=0.078]. During cocaine self-administration, the percent change of dopamine from baseline did not differ between the F344 and HIV Tg rats; however, the dopamine concentration for the HIV Tg rats was lower compared to the F344 rats.

Disclosures: S.E. Hemby: None. **S. McIntosh:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Title: Sex differences in the effects of dietary emulsifiers on physiology and behavior in mice

Authors: *M. K. HOLDER[1,2], B. CHASSAING[3], N. V. PETERS[2], J. WHYLINGS[2], A. T. GEWIRTZ[3], G. J. DE VRIES[2];
[2]Neurosci. Inst., [3]Inst. for Biomed. Sci., [1]Georgia State Univ., Atlanta, GA

Abstract: The internal and external surfaces of all mammals are colonized by a diverse group of microorganisms, collectively called the microbiota. The microbiota-gut-brain axis is known to influence brain development and social behavior. Recently, dietary emulsifiers, detergent-like molecules commonly used in processed foods such as ice cream, alter the composition of the intestinal microbiota and induce chronic low-grade inflammation, obesity, and metabolic syndrome in mice. Here, we tested whether emulsifier treatment would influence the expression of anxiety-like and social behaviors in male and female mice. Upon weaning, male and female C57Bl/6 mice were placed into a new cage with water or a 1% solution of either carboyxlmethylcellulose or polysorbate 80 in the drinking water. Body weights were recorded weekly and mice were subjected to a battery of standard anxiety tests (one test per week): open field, elevated-plus maze, light-dark box, marble burying, and the three-chambered sociability. In addition, the length of the colon and weights of the colon, spleen, liver, and adipose tissue were recorded at the end of the experiment. Emulsifier treatment altered anxiety-like behavior in male mice, as indicated by reduced time in the center of the open field test and increased distance travelled in the elevated plus maze. Emulsifier treatment did not affect anxiety-related behaviors in females; however, it reduced the preference for a novel conspecific mouse in the three-chambered sociability test in female, but not male, mice. Emulsifier treatment caused a 60-80% increase in adiposity and chronic intestinal inflammation, characterized by shortened colons, in both male and female mice compared to water-treated controls. Emulsifiers also increased total body weight of males, but not females. Taken together, these results indicate that emulsifier treatment leads to more severe physiological and behavioral alterations in male mice and acts on the neural circuits underlying social and anxiety-like behaviors.

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Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: Division of Intramural Research of the National Institute of Nursing Research of the NIH, Bethesda, Maryland

Title: The role of TRAIL in cancer-related fatigue following radiation therapy

Authors: *S. D. DETERA-WADLEIGH[1], L. R. FENG[2], L. N. SALIGAN[2];
[1]Human Genet. Branch, NIMH, [2]Natl. Insitute of Nursing Res., NIH, Bethesda, MD

Abstract: Chronic fatigue is one of the most common and debilitating side effects of cancer and cancer treatment, and yet its etiology remains elusive. The goal of this study is to understand the underlying inflammatory mechanism and identify co-occurring symptoms of chronic fatigue in non-metastatic prostate cancer men following radiation therapy (RT) completion. The initial investigation included 40 men scheduled to receive RT at the National Institutes of Health, Bethesda, Maryland. Data were collected before RT (T1) and one year after RT (T2). Fatigue was assessed using the Functional Assessment of Cancer Therapy-Fatigue questionnaire (FACT-F). Whole genome microarray and cytokine multiplex panel examined fatigue-related transcriptome and serum cytokine changes, respectively. The significantly changed cytokine from the initial investigation was validated using sera from 46 men two years after completing RT (T3) for prostate cancer at Georgetown University Hospital, Washington, DC. Further in vitro validation determined the effect of the significantly changed cytokine on cell viability as quantified by MTT assay. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL decoy receptor, *TNFRSF10C* (TRAIL-R3), were significantly upregulated (fold change=1.4, p=2.5x10^{-5}) in fatigued subjects (n=15) at T2. Cognitive deficits were also observed in fatigued subjects at T2. Further, TRAIL correlated with fatigue at T3 in a separate cohort. TRAIL caused selective cytotoxicity in neuronal cells, but not in microglial and muscle cells, in vitro. Late-onset inflammation directed by TRAIL may play a role in chronic fatigue pathogenesis in prostate cancer survivors. Selective vulnerability of neurons to TRAIL may contribute to chronic fatigue-related cognitive deficits.

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Poster

446. Neuroimmunology: Behavioral Effects

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Support: NIH Grant R01 MH082930

NIH T32 Grant AG00096

Title: Neuroimmune modulation of hippocampal synaptic signaling, neural circuit activity, and memory retrieval

Authors: T. E. WHITE[1], J. CZERNIAWSKI[2], G. LEWANDOWSKI[2], *J. F. GUZOWSKI[3]; [1]Neurobio. & Behavior, Univ. of California, Irvine, CA; [2]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [3]Dept Neurobiol/Behavior, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: Altered brain cytokine expression is at the core of many distinct neurological conditions that affect memory function and cognition. In order to develop therapies aimed at maintaining proper memory function in patient populations with these various conditions, it is critical to gain multilevel insight into how cytokines impact brain function and memory. Our previous studies show that mild acute neuroinflammation induced by systemic lipopolysaccharide (LPS) administration impairs context discrimination memory retrieval and disrupts pattern separation-like activity in hippocampal neuronal ensembles. Furthermore, blocking microglial activation systemically during LPS challenge with minocycline: i) abrogates increases in proinflammatory cytokines, ii) restores neural circuit activity in hippocampus, and iii) rescues context discrimination memory. However, systemic LPS-induced elevation of cytokines is brain-wide and not specific to the hippocampus. Therefore, we tested whether blocking microglial activation selectively within dorsal hippocampus (DH) is sufficient to rescue hippocampal neural circuit activity and context discrimination. Rats were trained to criterion in a context discrimination conditioning (CDC) task and received infusion of saline or minocycline (2 μg) directly into DH 5 min prior to administration of saline or LPS (i.p; 6 h before testing). Minocycline infused directly into DH in conjunction with LPS completely blocked context memory retrieval impairment in CDC. These preliminary data provide powerful evidence that local cytokine-mediated changes within DH are sufficient for the deficits in memory retrieval following peripheral immune stimulation. In order to determine how cytokines mediate alterations in neural circuit activity, and ultimately behavior, we are examining how LPS alters synaptic function and plasticity using transcriptomic and biochemical approaches. Cellular pathway analysis of RNA microarray data from whole DH indicates dysregulation of several signaling pathways critical to memory function including glutamatergic neurotransmission, calcium signaling, actin cytoskeleton regulation, and LTP. We are now analyzing synaptoneurosomal fractions of the dentate gyrus/CA3 and CA1 hippocampal subregions of candidate proteins in these pathways, including phospho-/total cofilin and phospho-/total GluR1. Together, this multilevel analysis will provide a framework for understanding how cytokines disrupt synaptic signaling and neural circuit dynamics in hippocampus necessary for context discrimination memory function.

Disclosures: T.E. White: None. **J. Czerniawski:** None. **G. Lewandowski:** None. **J.F. Guzowski:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: Nestlé

Title: Low grade colitis sensitizes sickness response to lipopolysaccharide in BALB/c mice

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Abstract: The gut has been proposed as a motor in critical illness driving multiple organ dysfunction (Mittal & Coopersmith, Trends Mol. Med., 2014). Indeed, mucus erosion, changes in intestinal microbial composition, decreased expression of tight junction molecules, and impaired epithelial renewal can all contribute to propagation of inflammation during critical illness. In the present study, we set out to determine if chronic low-grade gut inflammation exacerbates subsequent sickness behavior and brain cellular activation patterns in response to bacterial lipopolysaccharides (LPS). To do so, C57BL/6 and BALB/c male mice, which are prone and resistant respectively to dextran sodium sulfate (DSS)-induced chronic colitis (Melgar et al., Am. J. Physiol., 2005), were subsequently injected intraperitoneally with LPS or saline. Food intake and social exploration were assessed at regular intervals prior and after intraperitoneal injection to assess sickness behavior, characterized by reduced food intake and social exploration, during acute DSS-induced colitis, remission, and after LPS administration. A one-week episode of moderate colitis led to temporary loss of body weight compared to control in both strains. The following four-week remission period during which animals were exposed to a low concentration of DSS was associated with a transient decrease in social exploration, but not in food intake, in C57BL/6 mice. No changes in food intake or social exploration were observed in BALB/c mice during the remission period. Subsequent intraperitoneal administration of bacterial LPS resulted in an earlier reduction in food intake in BALB/c, but not in C57BL/6, mice exposed to a low concentration of gut irritant as compared to control animals without DSS. Intraperitoneal injection as such also reduced social exploration one hour later only in BALB/c that had experienced colitis as compared to control animals of the same strain without DSS. Quantification of the cellular activation marker c-Fos in the brainstem sections of these animals is currently ongoing to establish which structures were sensitized in their response to LPS after an episode of colitis and remission. Taken together, these findings indicate that signs of sickness behavior during colitis and remission do not necessarily predict the occurrence of more severe sickness behavior during subsequent acute systemic inflammation and, conversely, that chronic

exposure to gut irritants that does not lead to concomitant sickness behavior can nevertheless sensitize neuroimmune circuits and give rise to the accelerated appearance of sickness behavior during a later episode of acute systemic inflammation.

Disclosures: J. Konsman: None. **L. Chaskiel:** None. **R. Dantzer:** None.

Poster

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Topic: F.05. Neuroimmunology

Support: NIH R01 NS092388-01A1

NIH R21 MH107002-01A1

Title: Behavioral and physiological sex-differences following cardiac arrest/ cardiopulmonary resuscitation

Authors: *M. M. GAUDIER-DIAZ, A. H. HAINES, W. H. WALKER, II, N. ZHANG, R. J. NELSON, A. DEVRIES;
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Abstract: Cerebral ischemia, caused by cardiac arrest or stroke, is a leading cause of death and disability worldwide. Thus, it is important to find ways to prevent it, facilitate recovery, and improve the quality of life of survivors. This heterogeneous condition elicits a cytotoxic and inflammatory environment, which can subsequently affect behavior. Previous research in this area has focused primarily on male mice, possibly contributing to unsuccessful clinical trials and limited treatment options. The present study aims to investigate possible sex-differences in the behavioral and physiological responses to global cerebral ischemia. To test potential sex-differences, both female and male C57/Bl6 mice experienced a cardiac arrest/ cardiopulmonary resuscitation (CA/CPR) or a sham procedure, and 4 days later tested in an open field apparatus. Then, tissue was collected for gene expression and histological analysis. Following CA/CPR, female mice display hyperlocomotion; however, this ischemia-induced characteristic behavior was resolved in the males. As expected, cerebral ischemia increased the gene expression of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and inducible nitric oxide (iNOS). Specifically, the CA/CPR procedure led to a significant increase in the expression of TNFα, IL-6 and iNOS among female mice, whereas in male mice it increased the expression of TNF-α and IL-1β. The data support the existence of sex-differences in the behavioral and physiological

responses to CA/CPR, and suggest that increased IL-6 and iNOS might contribute to the unresolved hyperlocomotion observed in female mice. Certainly, consideration of sex-differences will be essential for the advancement of cerebral ischemia research and treatment strategies.

Disclosures: **M.M. Gaudier-Diaz:** None. **A.H. Haines:** None. **W.H. Walker:** None. **N. Zhang:** None. **R.J. Nelson:** None. **A. DeVries:** None.

Poster

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Research Centers in Minority Institutions Award RR-03037

Title: Environmental mold exposure alters the relationships between microglial morphology and behavior

Authors: *K. PAGE[1,2], C. MCDERMOTT[2], S. UVAYDOV[3], D. ALEBDY[4], R. PANG[5], C. F. HARDING[1,4], C. L. PYTTE[1,2];
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Abstract: Our labs are interested in the impact of environmental mold on brain and behavior. We use C57/BL6 mice to investigate the effects of exposure to the black mold, *Stachybotrus chartarum*. We have previously shown that mold inhalation increased microglial expression of the inflammatory cytokine IL-1beta in the hippocampus and caused deficits in hippocampal-dependent memory. Microglia have the capacity to move between morphological phenotypes and

respond to pathogens or injury by transitioning from a ramified state into a primed state or a motile amoeboid state. To better understand the functional implications of microglial phenotypes we asked whether microglial morphologies were related to spatial memory or anxiety and whether these relationships were altered by mold exposure. In particular, the role of primed cells is not well understood. Therefore, we were interested in whether primed phenotypes seem to play an intermediary role between ramified and amoeboid cell types. Mice were nasally exposed to either saline vehicle or mold in saline vehicle 3 times per week for 4 weeks. Behavior was assessed using the Morris water maze and elevated plus maze. Brains were processed and immunohistochemistry was performed to quantify numbers of Iba1+ microglia in the dorsomedial dentate gyrus. Iba1+ cells were categorized into 3 morphologies: ramified, primed, and amoeboid. We found that within the vehicle control group, there were no correlations between numbers of microglia within each category and measures of spatial memory. Within the mold-treated group, numbers of ramified cells were not correlated with spatial memory. As expected, numbers of amoeboid cells were correlated with worse spatial memory. Surprisingly, numbers of primed cells were correlated with better spatial memory. Among control mice, increased numbers of primed cells were highly correlated with increased anxiety, while numbers of amoeboid microglia were correlated with lowered anxiety, though more weakly. In contrast, in mold-exposed mice, there were no correlations between numbers of microglia in any category and measures of anxiety. Thus, the relationships between the three morphological phenotypes of microglia and behavior clearly differ. These findings support the idea that primed cells may function differently from both ramified and amoeboid cells, rather than simply being intermediate between ramified and amoeboid phenotypes. Second, the same phenotype is related to different behavioral outcomes depending on mold exposure. This suggests, for example, that primed cells in mold-exposed mice have different secretory profiles than primed cells in control vehicle mice.

Disclosures: **K. Page:** None. **C. McDermott:** None. **S. Uvaydov:** None. **D. Alebdy:** None. **R. Pang:** None. **C.F. Harding:** None. **C.L. Pytte:** None.

Poster

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Topic: F.05. Neuroimmunology

Support: NIH R21MH104280

NIH R01MH106553

Title: Effect of neonatal immune activation on the developing immune system and learning in juveniles

Authors: *B. OSBORNE, S. SOLOMOTIS, J. M. SCHWARZ;
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Abstract: Activation of the immune system in early development increases the risk for a host of disorders. Notably, some neuropsychiatric disorders (e.g. Autism and learning disorders) present a strong male bias with diagnosis occurring during juvenile development. However, there has been little investigation into the impact of factors such as sex or early-life immune activation on the development of the immune system or the ontogeny of cognitive processes, such as learning. Microglia are the resident immune cells of the brain that respond to infection by increasing the release of immune molecules. Microglia communicate with neurons and have the ability to significantly affect neural function and behavior. Previous studies demonstrate that males have significantly more microglia than females in the hippocampus, cortex and amygdala on postnatal day four (P4), suggesting that males may be more vulnerable to the long-term negative consequences of early-life immune activation than females. The current experiments examined the effect of neonatal immune activation (low dose of Escherichia coli; $1x10^6$ CFU/0.1mL/kg) on microglia function and behavior in male and female rats alone or following a second immune challenge (Lipopolysaccharide; 25µg/mL) during juvenile development. Experiment 1 investigated potential learning delays using the Context Pre-exposure Facilitation Effect (CPFE) Paradigm at P24 and P30. Experiment 2 examined proinflammatory cytokine gene expression at P24 and P30. Experiment 1 revealed no differences in learning at P24 between sex or any treatment groups and all animals could perform the task. At P30, males and females that were neonatally infected and received a second immune challenge showed a trend towards enhanced learning relative to all other groups and showed better learning relative to P24 animals. Results from experiment 2 revealed decreased inflammatory gene expression in the prefrontal cortex and hippocampus at P30 relative to P24; however, at P24 IL-1β in the hippocampus is exaggerated in neonatally infected males and females following LPS, which is not observed at P30. These data indicate that immune function is changing rapidly throughout juvenile development and early-life immune activation does alter microglia function and cognition in juveniles, but not in a sex-specific manner.

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Poster

446. Neuroimmunology: Behavioral Effects

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CUNY CIRG 1937 CFH&CLP

Research Centers in Minority Institutions Award RR-03037 to Hunter College

Title: The effects of doxycycline and mold exposure on neurogenesis and contextual memory

Authors: *N. C. ABREU[1], A. EL-RAZI[2], I. VORONINA[3], K. PAGE[5], A. LOPEZ[1], S. UVAYDOV[4], C. F. HARDING[2,5], C. L. PYTTE[1,5];
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Abstract: People who experience repeated exposure to moldy buildings report cognitive deficits, including learning and memory impairments. Our labs have shown that mold exposure results in brain inflammation, decreased hippocampal neurogenesis, and impaired contextual memory in a mouse model. The anti-inflammatory antibiotic, doxycycline, has been shown to decrease brain inflammation, increase hippocampal neurogenesis, and improve contextual memory. We therefore tested whether doxycycline mitigated the effects of mold exposure on neurogenesis and memory. Adult mice were intranasally instilled (3 times/week, 4 weeks) with either saline vehicle or spores of the black mold *Stachybotrys chartarum*. Half of each group received either doxycycline in their chow or a matched control diet. Mice were trained on a conditioned fear task to test hippocampal-dependent contextual memory. We used immunohistochemistry to visualize immature neurons, using an antibody to doublecortin (DCX). Immature neurons were quantified in the subgranular zone, granular cell layer, and hilus of the dentate gyrus. The density of immature neurons and measures of contextual memory did not differ across the four treatments. Vehicle mice treated with the doxycycline diet showed a positive correlation between numbers of DCX+ cells in the entire dentate gyrus and the strength of contextual memory. The more immature neurons, the better their memory. This relationship was seen at both 30 minutes and 24 hours after training and was driven by numbers of neurons in the granular cell layer. In contrast, mice exposed to both mold and doxycycline showed the reverse correlation between DCX+ cells and memory. The more immature neurons, the worse their contextual memory. Interestingly, this correlation was driven by neurons in the subgranular zone and hilus. This relationship was only seen in the 30-minute test, not when long-term memory was tested at 24 hours post-training. There were no correlations between immature neurons and contextual memory in mice fed the control diet without doxycycline, either with or without mold exposure. Thus, in mice treated with a doxycycline diet, more immature neurons corresponded to better contextual memory in vehicle mice, whereas contrary to expectation, more immature neurons were correlated with decreased memory in mold-exposed mice. We propose that mold exposure in doxycycline-treated mice negatively impacted the maturation, incorporation, or function of immature neurons, such that they were associated with impaired learning.

Disclosures: **N.C. Abreu:** None. **A. El-Razi:** None. **I. Voronina:** None. **K. Page:** None. **A. Lopez:** None. **S. Uvaydov:** None. **C.F. Harding:** None. **C.L. Pytte:** None.

Poster

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Support: CONACyT Grant CB-2013-01 221653

Title: Blockage of central interleukin 6 trans-signaling prevents predator stress in the mouse

Authors: I. GONZALEZ-NATERAS[1], F. MONTERO-AMEZCUA[1], R. CUEVAS-OLGUIN[1], E. ESQUIVEL-RENDON[1], J. VARGAS-MIRELES[1], C. GONZALEZ-DEL CARPIO[1], S. ROSE-JOHN[2], *M. ATZORI[3];
[1]Facultad de Ciencias, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico; [2]Dept. of BIochemistry, Christian Albrechts Univ., Kiel, Germany; [3]Univ. Autónoma de San Luis Potosí, San Luis Potosi, Mexico

Abstract: Evidence of the last decade has revealed a potential link between stress and immune system. In particular, elevation in the levels of the pro-inflammatory cytokine interleukin 6 (IL-6) has been related to a variety of neuropsychiatric conditions including schizophrenia, depression, and post-traumatic stress disorder and other anxiety-related disorders. The relationship between IL-6 and the onset stressed behavior is not yet completely understood. We and others have already shown that an important mechanism of action of central IL-6 is through the so-called trans-signaling, which operates on all nucleated cells, including neurons. In order to assess the involvement of IL-6 trans-signaling in stress-related symptoms we used a transgenic mouse in which central IL-6 signaling was blocked by overexpression of a dimerized soluble version of the IL-6 transducer soluble glycoprotein 130 (sgp130Fc) in astrocytes, through the promotor of the astrocyte marker glial fibrillary acidic protein (C57BL/6 GFAP-sgp130Fc +/+, TG mice). Stress was induced by exposure to two session of one hour each in the presence of a domestic cat (*Felis Catus*) on two consecutive days. Stress levels were assessed measured by comparing the results of Porsolt forced swimming test (PS) and the Tail Suspension (TS) test. Five wild-type (WT) and six C57BL/6 GFAP-sgp130Fc transgenic (TG) mice were used for the experiment. Paired or unpaired Student-t test were used depending on the groups compared. As expected, WT mice were sensitive to stress (PS Latency to Immobility (LtI) = 155 ± 30 unstressed vs. 61 ± 7 s stressed, $P < 0.05$; PS PS First Two Minute Immobility Time (FTMIT) = 11 ± 6 vs. 45 ± 7, $P < 0.01$, same sample; TS LtI = 80 ± 10 unstressed vs. 48 ± 9 stressed; n = 5).

More importantly, non-stressed TG animal displayed a lower PS LtI (73 ± 11 s, n = 6) compared to WT animals but similar to stressed WT as well as to stressed TG (99 ± 10 s). Complementary to the PS LtI, unstressed TG animals displayed a PS FTMIT of 31 ± 7, higher than unstressed WT but not different from stressed WT or stressed TG (18 ± 6 s). As expected for WT, the TS LtI was longer for unstressed vs. stressed animals (80 ± 10 vs. 48 ± 9 s), and TS FTMIT was shorter for unstressed vs. stressed animals (13 ± 4 vs. 41 ± 7 s). Interestingly, TG animals displayed no sensitivity to the TS test, as their unstressed TS LtI (92 ± 23 s) did not differ from unstressed WT, nor from stressed TG (96 ± 20 s). Consistently with the TS LtI, unstressed TG animals' TS FTMIT (16 ± 4 s), did not differ from unstressed WT, not from stressed TG animals' FTMIT (16 ± 6 s). Altogether, these data suggest that central IL-6 trans-signaling is involved in the effects of predator stress in the rodent.

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Poster

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Topic: F.05. Neuroimmunology

Title: Psychomotor activation levels in mice are regulated by vasoactive intestinal peptide produced by bone marrow derived blood cells

Authors: *R. PANJWANI[1], C. R. GIVER[2], J. P. SCHRODER[3], J. FELGER[4], D. G. STEIN[5], E. K. WALLER[2];
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Abstract: Produced by both neurons and lymphocytes, vasoactive intestinal peptide (VIP) is a 28-amino acid neuropeptide that decreases Th1 and anti-viral immunity. VIP also plays a role in regulating neurological behavior; previous investigations have determined that VIP/peptide histidine isoleucine knockout (VIP-KO) mice exhibit altered circadian rhythms, decreased mobility, and selective cognitive disabilities. The goal of this study was to understand the role of VIP in murine behavior and determine whether VIP produced by bone marrow-derived blood cells influence neurological function. This question was addressed using radiation chimeras of

female C57BL/6J VIP-KO and wild-type (WT) mice receiving syngeneic transplants of bone marrow derived cells from either VIP-KO or WT donors. We hypothesized that the behaviors of VIP-KO mice will be changed to resemble wild-type mice in radiation chimeras engrafted with hematopoietic cells from WT mice and the behavior of wild type mice will be changed to resemble VIP-KO mice in radiation chimeras of WT mice engrafted with hematopoietic cells from VIP KO mice. Mice were observed for circadian rhythms, examined during forced swim tests (FST), and tested in contextual fear conditioning (CFC) paradigms 10-weeks post-transplant. Our hypothesis was supported and a difference was noted between transplant groups receiving WT and KO hematopoietic cells. Chimeras receiving KO bone marrow-derived cells exhibited significantly greater novelty induced locomotion. Wild type chimeras engrafted with KO cells showed significantly greater dark phase activity and swam significantly more during the FST. No difference was measured in the freeze response among radiation chimeras during the CFC. These findings indicate bone marrow-derived blood cells influence behavior and that VIP produced by blood cells is important in psychomotor activation levels. Future investigations include repeating experiments with male models and analyzing VIP content and brain histology to determine neurological function during behavioral testing.

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Poster

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Topic: F.05. Neuroimmunology

Support: Project Sponsor: Janssen R & D, LLC

Title: Targeted deletion of $P2X_7$ ion channels prevent Mycobacterium bovis, BCG induced depressive like behaviors in mice.

Authors: *J. C. O'CONNOR[1,2], L. REDUS[1], N. DERECKI[3], A. BHATTACHARYA[3]; [1]Pharmacol., UTHSCSA, San Antonio, TX; [2]Audie L. Murphy VA Hosp., San Antonio, TX; [3]Janssen Res. & Development, LLC, San Diego, CA

Abstract: Inflammation is implicated as a pathogenic factor in the development of depression symptoms. In mice, peripheral inoculation with the attenuated BCG strain of *Mycobacterium bovis* precipitates a depression-like behavioral phenotype due to persistent upregulation of peripheral cytokine expression, increased kynurenine metabolism and central

neuroinflammation. However, the CNS receptor-mediated mechanisms that contribute to BCG-induced depression-like behaviors are not fully understood. ATP-gated ion channels are preferentially expressed by brain microglia and contribute to neuroinflammation. We sought to determine whether $P2X_7$ contributed to the development of depression-like symptoms following BCG challenge in mice. Young adult male C57BL6/J (WT) mice or $P2X_7$ receptor null mice ($P2X_7^{-/-}$Jackson Laboratory) were inoculated with 10^7 colony-forming units of BCG or an equal volume of vehicle. All BCG challenged mice exhibited a 10%-12% reduction in body weight that steadily returned toward baseline levels by 10 days post BCG. Beginning two weeks post-BCG, mice were subjected to a series of behavioral tests spanning multiple discrete domains of depression-like symptomology. Sucrose preference was significantly reduced in BCG-challenged WT mice, and while baseline preference was slightly lower in $P2X_7^{-/-}$ mice, BCG did not reduce preference compared to vehicle treated $P2X_7^{-/-}$ controls. Similarly, BCG caused a reduction in spontaneous alternations in the Y-maze, increased time spent in the central area of an open field and reduced social interaction with a novel conspecific in WT mice, but in each of these tests $P2X_7^{-/-}$ mice were protected from BCG-induced behavioral changes. In contrast to previous reports, BCG did not increase immobility during the tail suspension test, but $P2X_7^{-/-}$ mice exhibited a general antidepressant phenotype. BCG-induced upregulation of the peripheral cytokine/chemokine response and splenomegaly were not dependent on $P2X_7$ gene deletion. Taken together, these data suggest that $P2X_7$ may play an important role in mediating the depression like behavioral effects of chronic peripheral inflammation induced by BCG.

Disclosures: **J.C. O'Connor:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Research & Development, LLC.. **L. Redus:** None. **N. Derecki:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **A. Bhattacharya:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC..

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: NIH Grant DA034721

Title: Astrocyte modulation of stress-enhanced fear learning, an animal model of post-traumatic stress disorder

Authors: *M. E. JONES[1], L. B. COOPER[2], E. B. KELLY[2], J. E. PANICCIA[2], C. L. LEBONVILLE[2], D. T. LYSLE[2];
[1]Psychology and Neurosci., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Neuroimmune signaling is important in learning and memory processes. Further, converging evidence suggests that neural immune interactions, including astrocyte activity, are altered following stress exposure. For example, our laboratory has shown that the severe stressor in stress-enhanced fear learning (SEFL), an animal model of post-traumatic stress disorder (PTSD), induces a time-dependent increase in interleukin-1β (IL-1β) protein and mRNA and that blocking IL-1 signaling prevents the development of SEFL. Here, we employed double labeling fluorescence immunohistochemistry to determine the cellular source of IL-1β and took advantage of recent advances in chemogenetic technology to begin to explore the role of astrocytes in SEFL. Specifically, we used glial DREADDs (designer receptors exclusively activated by designer drugs) to test whether Gi signaling in hippocampal astrocytes modulates the development of SEFL.
In Experiment 1, rats were exposed to Context A of the typical SEFL paradigm (15 2mA scrambled foot shocks over 90 minutes on a 6 minute variable interval schedule) and sacrificed via transcardial perfusion 48 hours later. Brains were extracted and tissue was processed for immunohistochemistry with primary antibodies against IL-1β and cell type specific markers for astrocytes (GFAP), neurons (NeuN), and microglia (Iba-1). Alexa fluor -conjugated secondary antibodies were used for visualization. Confocal microscopy analyses revealed significant co-localization of IL-1β and GFAP in the dorsal hippocampus in animals exposed to the stressor.
In Experiment 2, rats were infused with *AAV8-GFAP-hm4di-mcherry* directly into the dorsal hippocampus and allowed three weeks to recover from surgery and for the virus to express. Rats were then exposed to the typical SEFL paradigm and administered Clozapine-n-oxide (CNO; 3 mg/kg, s.c.) immediately, 24h, and 48h after removal from Context A. Preliminary data showed that CNO significantly attenuated fear learning to Context B on test day 1.
In summary, Experiment 1 showed that astrocytes are the predominant cellular source of stress-induced IL-1β in the dorsal hippocampus. Strikingly, our preliminary data in Experiment 2 showed that activating astroglial Gi signaling attenuated SEFL, a PTSD-like phenotype. Further studies in our laboratory are testing the effect of activating astroglial Gq signaling following the severe stressor on SEFL and whether any effects of glial GPCR signaling occur through an IL-1β-dependent mechanism.

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Poster

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Topic: F.05. Neuroimmunology

Title: Sustained peripheral inflammation triggers anandamide hydrolysis to promote anxiety.

Authors: *H. A. VECCHIARELLI[1,2], M. MORENA[2], M. STICHT[2], C. M. KEENAN[2], W. HO[2], K. A. SHARKEY[2], M. N. HILL[2];
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Abstract: There is a high degree of comorbidity between chronic peripheral inflammatory diseases and neuropsychiatric disorders, such as anxiety and depression. However, the mechanisms linking the two have not been fully elucidated. The endogenous cannabinoid system is known to regulate both inflammation and anxiety, making it a candidate system to investigate interactions between the two. This study's aim was to determine if chronic peripheral inflammation, in the form of colitis, alters central endocannabinoid levels and to determine if these changes related to inflammation-induced anxiety. Colitis was induced by administration of trinitrobenzene sulfonic acid (TNBS) intracolonically to adult, male, Sprague Dawley rats. Seven days after the induction of colitis, anandamide levels are reduced in the amygdala (-30%), hippocampus (-14%) and medial prefrontal cortex (-30%). In contrast, 2-arachidonylglycerol (2-AG) levels are increased in the hippocampus (16%) and medial prefrontal cortex (21%). We observed an increase in anxiety behaviors following colitis (approximately a 50% reduction in open arm time using an elevated plus maze), with no change in locomotor activity. This inflammation-induced anxiety was reversed by acute intracerebroventricular (ICV) administration of the fatty acid amide hydrolase (FAAH) inhibitor PF-4458945 (100 µg), which increases anandamide levels. These findings increase our understanding of the mechanisms underlying anxiety behaviors in chronic peripheral inflammatory states. They suggest that similar to stress-induced anxiety, inflammation-induced decreases in anandamide signaling are likely relevant for the change in emotional behaviors associated with chronic inflammation.

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Poster

446. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 446.17/DDD11

Topic: F.05. Neuroimmunology

Title: Genipin attenuates LPS-induced persistent changes of emotional behaviors and neural activation in the hypothalamic paraventricular nucleus and the central amygdala nucleus

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Abstract: Sickness behavior is a series of behavioral and psychological changes that develop in those stricken with cancers and inflammatory diseases. The etiological mechanism of sickness behavior is not known in detail, and consequently there are no established standard therapies. Administration of the bacterial endotoxin lipopolysaccharide (LPS) induces sickness behavior in rodents. Genipin, an aglycon derived from an iridoid glycoside geniposide extracted from the fruit of Gardenia jasminoides, has anti-inflammatory and antidepressant activities. However, the effects of genipin on inflammation-induced changes in emotional behaviors are unknown. In this study, we examined the effects of genipin on LPS-induced inflammation in BV-2 cells and sickness behavior in mice. Pretreatment with genipin inhibited LPS-induced increases in NO production and reduced the mRNA levels of inflammation-related genes (iNOS, COX-2, IL-1β and IL-6) in BV-2 cells. Oral administration of genipin ameliorated LPS-induced depressive-like behavior in the forced swim test and social behavior deficits 24h after LPS administration in mice. LPS-induced expression of mRNAs for inflammation-related genes and the number of c-fos immunopositive cells decreased in the paraventricular nucleus (PVN) of the hypothalamus and the central nucleus of the amygdala (CeA), suggesting that genipin attenuates LPS-induced changes of emotional behaviors through inhibition of neural activation and inflammatory responses in the PVN and CeA. In conclusion, KKT ameliorated LPS-induced sickness behavior *via* suppression of neural activation in the PVN and CeA, but had little effect on LPS-induced inflammation. These results indicate that KKT has potential for treatment of sickness behavior. Our findings also suggest that suppression of neural activation in the PVN and CeA is a potential therapeutic approach for treatment of sickness behavior.

Disclosures: **T. Yabe:** None. **Y. Hiraki:** None. **S. Nishida:** None. **R. Araki:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: Intra-mural funds, Tata Institute of Fundamental Research, Mumbai, India

The Indian Council of Medical Research, Government of India

Lady Tata Memorial Trust, Mumbai, India

Title: A history of juvenile malaria predisposes mice towards anxiety-like responses, neuroinflammation, and decreased neurogenesis in response to adult-onset stress

Authors: *S. K. GUHA, I. SARKAR, S. SHAH, M. PATGAONKAR, S. SHARMA, S. PATHAK, V. A. VAIDYA;
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Abstract: Children residing in malaria endemic regions are particularly susceptible to malaria. Also, this early-life window is a critical-period for development and maturation of the nervous system. Exposure to inflammatory insults during this critical-period has life-long neuropsychiatric consequences. We therefore asked whether a history of juvenile mild malaria predisposed individuals towards mood-related disorders and neurocognitive deficits.
We employed a mouse model of juvenile mild malaria, and combined it with a four-week chronic unpredictable mild stress (CUS) regime during adulthood to examine latent behavioural, neuroimmune, and neurogenic consequences of the infection.
Adult mice with a history of juvenile malaria exhibited an early-onset anxiety-like behaviour in open field test and elevated plus maze when exposed to a CUS regime. These mice also had elevated stress associated serum cytokine response and microglial activation within the hippocampal neurogenic niche. Furthermore, there was a concomitant decline in adult hippocampal neurogenesis. Interestingly, while the total number of immature neurons remained the same, baseline proliferating progenitor numbers within the adult hippocampal neurogenic niche had significantly declined in mice with a history of juvenile malaria.
In conclusion, history of juvenile mild malaria predisposes individuals towards post-stress anxiety-like responses in adulthood, which is strongly correlated with both neuroinflammatory and neurogenic changes. These findings lead credence to the idea that the burden of malaria in early-life may result in sustained CNS changes that get unmasked later in life by subsequent insult(s).

Disclosures: **S.K. Guha:** None. **I. Sarkar:** None. **S. Shah:** None. **M. Patgaonkar:** None. **S. Sharma:** None. **S. Pathak:** None. **V.A. Vaidya:** None.

Poster

446. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: Whitehall Foundation Research Grant

R01 GM107469

R21 AG048410

Title: CX_3CR1 -expressing monocytes alter learning and learning-dependent dendritic spine plasticity during viral immune activation

Authors: ***J. M. GARRE**[1], H. MOURA-SILVA[2], J. J. LAFAILLE[2], G. YANG[1];
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Abstract: Peripheral infections often cause behavior changes and cognitive impairment. The activation of innate immune system is the primary and immediate defense by which an organism copes with infection. How infection-induced innate immune response modulates synapse plasticity and learning is unknown. In this study, we used transcranial two-photon microscopy to examine the remodeling of postsynaptic dendritic spines in the living mouse cortex after systemic administration of a synthetic double-stranded RNA, which mimics the acute phase of viral infection. We find that viral-like immune activation has potent effects on dendritic spine dynamics, increasing dendritic spine elimination and decreasing learning-dependent spine formation. Concomitantly, mice show deficits in multiple learning tasks during viral immune activation. Using in vivo cell depletion, we show that these synaptic alterations in the cortex are mediated by peripheral CX_3CR1-expressing monocytes, but not by brain-resident microglial cells. Furthermore, inhibiting TNFα production in monocytes prevents the abnormal spine remodeling and protects mice from learning deficits. Together, our study identifies CX_3CR1^+ monocytes and TNFα as key immune components that drive synaptic alterations and cognitive dysfunction during systemic infection.

Disclosures: **J.M. Garre:** None. **H. Moura-Silva:** None. **J.J. Lafaille:** None. **G. Yang:** None.

Poster

446. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 446.20/DDD14

Topic: F.05. Neuroimmunology

Support: Stanley Medical Research Institute

Title: Toxoplasma gondii infection and blunted response to stimulant drug administration

Authors: *R. MCFARLAND[1], M. V. PLETNIKOV[2], R. H. YOLKEN[3];
[1]molecular microbiology and immunology, [2]Behavioral Neurosci., [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Infection with the neurotropic parasite *Toxoplasma gondii* has long been identified as a risk factor in the development of human psychiatric disease, particularly schizophrenia. The induction of neuro-immune responses may be behind the link between the parasite and disease, and is consistent with the most recent advances in human genetic association studies. These studies indicate changes to immune mechanisms as strong genetic risk factors for the development of schizophrenia. We have established a mouse model system that has a strong and consistent behavioral phenotype of infection. In this model we see that long-term infection with Toxoplasma creates a severely blunted response to the dopaminergic stimulant drugs cocaine and amphetamine, suggesting that the parasite is inducing brain changes which are relevant to dopamine release. The profound inhibition of the biological effects of these stimulants is the strongest and most consistent behavioral effect yet attributed to latent infection with the parasite, and one that is directly relevant to human brain function as well.

Disclosures: R. McFarland: None. **M.V. Pletnikov:** None. **R.H. Yolken:** None.

Poster

446. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 446.21/EEE1

Topic: F.05. Neuroimmunology

Title: Vium Digital Vivarium™ enables automated drug efficacy assessment in animal models of multiple sclerosis

Authors: *L. SCHAEVITZ, D. FORD, M. LIM;
Vium, San Mateo, CA

Abstract: Current methods for screening and evaluating new therapeutics in rodent disease models are not only labor-intensive, but are also confronted by several challenges, including the ability to consistently produce reproducible and unbiased data. To address these limitations, we created the Vium Digital Vivarium™, which monitors animals continuously for disease and health-related endpoints, without human interaction, to evaluate drug efficacy. Our intelligent sensor and camera network not only monitors health endpoints, including overall activity, breathing rate, and circadian rhythms but also stores the data in auditable electronic and videographic records. As a proof of principle, we conducted two standard studies using the mouse Myelin Oligodendrocyte Glycoprotein $(MOG)_{1-125}$ induced experimental autoimmune encephalomyeltitis (EAE) model of multiple sclerosis (MS) in the Vium Digital Vivarium. Traditionally, MS disease onset and progression in this mouse model are evaluated using Disease Activity Index scoring (DAIs), a scale-based subjective scoring system, which monitors clinical signs of two motor functions, abnormal gait and paralysis, to assess severity of the disease. Data from our platform showed significant changes in overall activity, which preceded disease onset as measured by traditional DAIs scoring. Given the clear relationship between activity and the DAIs, we are using machine learning to develop an algorithm to automatically identify disease onset and severity in MS. The DAIs and metrics from the first standard study are being used to train classifiers, while data from the second study, including standard of care drugs FTY720 and methylprednisolone, will be used to test performance of the algorithm. Here we demonstrate how a low-touch digital platform can pave new ways for more rapid, reproducible, drug discovery and evaluation, not only for MS, but also more broadly in other autoimmune diseases including Lupus as well as neurodegenerative diseases such as Parkinson's, Huntington's, and ALS.

Disclosures: **L. Schaevitz:** A. Employment/Salary (full or part-time): Vium. **D. Ford:** A. Employment/Salary (full or part-time): Vium. **M. Lim:** A. Employment/Salary (full or part-time): Vium.

Poster

446. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 446.22/EEE2

Topic: F.05. Neuroimmunology

Support: NIH Grant R21MH105826

OSU Fellowship

Title: Early life depletion of microglia programs lifelong mood-related behavior and brain development

Authors: *L. H. NELSON[1], S. WARDEN[2], K. M. LENZ[3];
[1]Ohio State Univ., Columbus, OH; [2]Dept. of Psychology, [3]Dept. of Psychology and Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Microglia, the brain's resident immune cells, regulate normal processes of brain development, including synaptic pruning, axon outgrowth, proliferation, and progenitor pool size (Schafer et al., 2012 Neuron; Squarzoni et al., 2014 Cell Reports; Cunningham et al., 2013 J Neurosci; Shigemmoto-Mogami et al, 2014 J Neurosci). Perinatal perturbations such as early life stress (ELS) increase microglial activation and alter mood-related behavior (Roque et al., 2012 Brain Behav Immun; Wigger et al., 1999 Physiol Behav), but it is unknown if microglia influence the basal development of mood-related behavior or brain circuits. The neonatal male brain has more activated microglia than the female brain (Lenz et al., 2013, J Neurosci; Schwarz et al., 2012 J Neurochem), and males are generally more sensitive to early life perturbations, but whether microglia contribute to sex differences in the development of mood related behavior is also unknown. We hypothesized that microglia contribute to early life programming of mood-related behavior, and used a microglial depletion strategy to test this hypothesis in male and female rats. We used central infusion of liposomal clodronate (2μL icv; Encapsula Nanoscience) on postnatal day (P) 1 and P4 to induce microglial apoptosis during the neonatal period. We analyzed the depletion and recolonization of microglia via densitometry of Iba1 immunostaining. Approximately 90% of microglia were depleted brain-wide by P2, and microglia recolonized the brain at P12. Following microglial depletion, animals were grown to adolescence or adulthood for behavioral testing, including anxiety tests (the elevated plus maze and open field), behavioral despair testing (forced swim test), and HPA axis tone following acute restraint stress. We found that clodronate treated rats had lower anxiety as adolescents and adults, decreased despair-like behavior as adults, and lower restraint-evoked corticosterone levels in females, with few sex differences observed. These results suggest that microglia are important for the developmental programming of mood-related behavior. We are currently using Golgi-Cox staining to and single cell reconstruction to assess if neonatal microglial depletion impacts dendritic spine density in the amygdala, hippocampus, prefrontal cortex, and bed nucleus of the stria terminalis. Future research will determine how ELS affects microglial function during development, and whether microglial depletion is protective against the programming effects of ELS on adult mood-related behavior. These studies give important insight into the role microglia play in normal development of brain and behavior.

Disclosures: **L.H. Nelson:** None. **S. Warden:** None. **K.M. Lenz:** None.

Poster

446. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 446.23/EEE3

Topic: F.05. Neuroimmunology

Title: Attenuation of TLR4 signalling prevents behavioural alterations induced by a short alcohol binge during adolescence

Authors: *J. W. JACOBSEN[1], F. BUISMAN-PILJIMAN[1], D. BARRATT[1], S. MUSTAFA[1], M. R. HUTCHINSON[2];
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Abstract: Background
Adolescence is a crucial neurodevelopmental period. During this stage, brain regions governing novelty and pleasure seeking are still immature. This increases the propensity for adolescence to engage in risky behaviours such as, binge drinking. Unfortunately, these same brain regions are susceptible to alcohol. Consequently, binge drinking can perturb neurodevelopment causing detrimental consequences in adulthood. However, current animal models are limited in their portrayal of the human condition nor do they consider the role of immune signalling. Therefore, the aims of this experiment were to develop a better animal model of adolescent alcohol exposure and to characterise its effects on immune signalling and later life alcohol-related behaviours.
Methodology
Between postnatal days 22 and 25, balb/c mice received an oral gavage of alcohol (0.5 – 3.5g/kg) daily, replicating a short alcohol binge paradigm. Control mice received volume-matched gavages of saline. Mice were weaned (P30), separated in to single sex cages and allowed to mature undisturbed until adulthood (P56 – 70). Mice subsequently underwent elevated plus maze (anxiety), conditioned place preference (alcohol wanting/seeking) and "drinking in the dark" (binge drinking).
Results
Four doses of alcohol during adolescence dose dependently increased alcohol-induced conditioned place preference ($p<0.0001$) in adults. Further, compared to saline controls, adolescence alcohol exposure augmented alcohol intake in adults ($p=0.018$). However, this model did not effect basal elevated plus maze times ($p>0.05$). Subsequent analysis of nucleus accumbal mRNA, revealed an increased expression of TLR4-related mRNAs in mice who received alcohol during adolescence compared to saline ($p<0.01$).
To further elucidate the role of TLR4, (+)-Naltrexone, an antagonist was administered 30mins before or after the adolescent binge paradigm. When tested in adulthood, (+)-Naltrexone treated animals had attenuated binge alcohol consumption later in life ($p<0.0001$), but did not alter other

responses.

Conclusions

This study highlights that even a small amount of alcohol, when given during a critical neurodevelopmental period, can potentiate alcohol wanting/seeking behaviour and alcohol intake later in life. Interestingly, attenuation of TLR4 before or after adolescent exposure reduced only binge alcohol intake. Given that these behaviours are governed by distinct brain regions, it suggests, a regional specific role for TLR4.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.01/EEE4

Topic: F.07. Autonomic Regulation

Support: Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office

Title: Robust continuous estimation of cardiac output and systolic time intervals using a moving ensemble method

Authors: *M. CIESLAK[1], W. S. RYAN[2], W. MEIRING[3], V. BABENKO[4], H. ERRO[4], Z. M. RATHBUN[4], J. BLASCOVICH, PhD.[2], S. T. GRAFTON[2];
[1]Psychological and Brain Sci., UCSB, Santa Barbara, CA; [2]Psychological and Brain Sci., [3]Statistics and Applied Probability, [4]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Impedance cardiography (ICG) is a non-invasive method used to measure cardiac output and systolic time intervals during psychophysiology experiments. Autonomic responses to motivation, stress and task engagement can be captured using this technique. However, ICG practitioners currently must choose between two extremes of analyzing beat-by-beat signals or methods that reduce each epoch of data into a single ensemble averaged heartbeat. Beat-by-beat measurements are contaminated with respiratory artifacts and random noise, while ensemble averaging is unable to capture transient changes that occur within an epoch. Here we describe a recently developed moving ensemble method that combines the noise-removing aspects of ensemble averaging while maintaining the ability to detect transient changes in cardiac output and systolic time intervals. Open source software for applying the moving ensemble method is provided.

Study 1 recorded ICG data from 30 individuals during two repetitions of a cold pressor. Study 2 acquired ICG data from two individuals over four days where multiple tasks were run in a counterbalanced order. Tasks included a valsalva maneuver, a cold pressor and a video game. We show that the moving ensemble method captures fast changes in cardiac output and systolic time intervals that are missed by fixed-window ensemble averaging.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.02/EEE5

Topic: F.07. Autonomic Regulation

Title: The NeuroCorrelates of steady state blood pressure and heart rate in healthy humans

Authors: *L. BARNDEN[1], R. BURNET[2], P. DEL FANTE[4], R. KWIATEK[5], B. CROUCH[3], Z. SHAN[1];
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Abstract: In healthy humans, normal inter-subject variation in steady state Heart Rate (HR) and Blood Pressure (BP) may be mediated by normal variability in the brain nuclei that control autonomic nervous system activity. The neurocorrelates of HR and BP have been investigated under mental and emotional stress, but not under steady state conditions. In this cross-sectional study, SPM5 voxel-wise regressions were performed between structural brain MRI levels and steady state BP and HR in 23 subjects. MRI included grey matter volume (GMvol) and white matter volume (WMvol) images from voxel based morphometry, and T1- and T2-weighted spin-echo (T1w and T2w) images. Steady state systolic BP (sysBP), diastolic BP (diaBP) and pulse pressure (PP), and HR, all in two postures (erect and reclining) were extracted from 24 hour BP monitoring. Voxel-wise MRI regressions were adjusted for age and the appropriate global value. Significant clusters for MRI vs BP or MRI vs HR were identified via corrected cluster P (ccP) after imposing a false discovery rate (FDR) of 0.05 to account for the 64 regressions performed. Correlations between pairs of the HR and BP measures were significant between the erect and reclining values for each measure, and between all BP measures in the erect posture and in the reclining posture (except between erect diaBP and erect PP), but not between HR and any BP. In the voxel-wise analysis, FDR < 0.05 was satisfied for ccP < 0.005. We identified 6 significant

clusters, all from WMvol regressions, with three from each posture. WMvol associations occurred in the right prefrontal cortex (erect and reclining HR), the medial prefrontal cortex (reclining sysBP and reclining diaBP) and the caudal pons - inferior cerebellar peduncle (erect sysBP and erect diaBP). In a healthy population, normal variability in relative local WM volume contributes to the normal variability in BP and HR.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

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Program#/Poster#: 447.03/EEE6

Topic: F.07. Autonomic Regulation

Title: Protective effects of training status on autonomic modulation and blood pressure during acute exercise in normotensive adults and elderly

Authors: *A. S. ZAGO, G. F. M. MARTINS, L. P. BARBOSA, L. L. CESAR, A. M. JACOMINI, R. F. SILVA, S. L. AMARAL;
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Abstract: Increases in sympathetic activity (SA) and reductions in vagal activity may be considered one of the causes for the high incidence of hypertension (HT) in elderly. In the opposite way, regular exercise training has the capacity to reduce blood pressure (BP) by improving cardiac autonomic modulation. However, increases in SA observed during acute exercise may cause a huge increase on BP, exposing the participant to any cardiovascular events. Although it is well established at the literature, little is known about the effects of a good level of training status (TS) in preventing the high elevation of BP during exercise. The purpose of this study was to evaluate the cardiac autonomic modulation and BP in normotensive adults and elderly with different levels of TS during the rest and acute exercise. Adults (n=14, 54±8 years old) and elderly (n=21, 70±5 years old) with no cardiovascular disease performed an incremental test on treadmill to estimate maximal oxygen uptake (VO_2max). Blood pressure and heart rate variability (HRV) were measured during acute exercise. Autonomic modulation was evaluated by normalized low (LFnu) and high-frequency (HFnu) components of the R-R variability accessed by RS800 heart rate monitor (polar®) during rest (10min) and acute exercise (40 min walking at 50-60% of VO_2max). All participants were divided according to VO_2max in TS1 (low VO_2max) and TS2 (high VO_2max) according to American Heart Association. In the adults group, differences were found in LF (TS1=85±5 vs TS2=61±24 nu, $p<0.02$) and HF (TS1=14±2

vs TS2=38±5 nu, p<0.03) at rest moment. Besides these different results, systolic (SBP) and diastolic BP (DBP) were similar between both groups (TS1=112±6/78±6 and TS2=113±9/74±7 mmHg). During exercise, no significant changes were observed in LF (TS1=70±8 vs TS2=77±10 nu), HF (TS1=29±3 vs 27±3 nu) or BP (TS1=137±8/76±5 and TS2=144±14/77±9 mmHg) between groups. In the elderly group, no differences were found for LF (TS1=39±6 vs TS2=57±8 nu) and HF (TS1=59±12 vs TS2=42±8 nu) either at rest or during exercise (LF - TS1=55±14 vs TS2=70±6 and HF - TS1=44±14 vs TS2=29±6 nu). Blood pressure was similar between groups at rest (TS1 = 113±8/62±8 and TS2 = 112±15/63±11 mmHg), however during acute exercise the DBP was lower in TS2 compared with TS1 (TS1=137±11/80±4 and TS2=137±14/70±9 mmHg, p<0.05). These results suggest that normotensive individuals, with better training status, can perform a low intensity acute exercise with similar increase of cardiac autonomic balance and smaller increase of DBP, compared with lower training status, even though walking at higher speed on treadmill, which may contribute to avoid cardiovascular events during exercise.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

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Topic: F.07. Autonomic Regulation

Support: NIH Grant R01 HL-113251

NIH Grant R01 NR-015038

Title: Cognitive and mood deficits and associations between symptoms and disease severity in early-diagnosed, treatment-naïve obstructive sleep apnea

Authors: L. EHLERT[1], B. ROY[2], D. KANG[3], M. WOO[2], R. AYSOLA[4], *R. KUMAR[1,5,6,7]; [1]Dept. of Anesthesiol., [2]Sch. of Nursing, [3]Dept. of Med., [4]Dept. of Pulmonary Med. and Critical Care, [5]Brain Res. Inst., [6]Radiological Sci., [7]Bioengineering, Univ. of California at Los Angeles, Los Angeles, CA

Abstract: Obstructive sleep apnea (OSA) is a serious health condition accompanied by repeated blockage of upper airway, with continuous diaphragmatic efforts to breathe during sleep. Brain regions with structural changes emerge in early-diagnosed, without-treatment OSA subjects in

areas that control cognitive and mood functions. However, the status of cognitive and mood functions and relationship between those symptoms and disease severity in early-diagnosed, treatment-naive OSA subjects is unclear. Our aim was to examine cognitive and mood functions and associations between cognitive and mood scores to disease severity [oxygen saturation nadir (SaO_2 nadir) and apnea hypopnea index (AHI)] in recent-diagnosed, non-treated OSA subjects. We studied 21 early-diagnosed, treatment-naïve OSA (age, 48.4±10.7 years; body mass index, 31.2±5.0 kg/m^2; AHI, 36.5±20.7 events/hour; 18 male) and 19 control subjects (age, 52.8±10.8 years; body mass index, 26.5±3.5 kg/m^2; 15 male). Cognitive functions were examined with the Montreal Cognitive Assessment (MoCA) test, depressive symptoms with the Beck Depression Inventory II (BDI-II), and anxiety symptoms with the Beck Anxiety Inventory (BAI). MoCA values < 26, and BDI-II and BAI values >10 are considered abnormal scores. The AHI and SaO_2 nadir values were derived from overnight polysomnography study of all OSA subjects. No significant differences in age or gender appeared between OSA groups. However, OSA subjects had significantly higher body mass index over controls. MoCA scores were significantly reduced in OSA compared to controls (OSA vs controls; 25.9±2.8 vs 27.4±1.7, p = 0.04). BDI-II (6.6±7.4 vs 1.7±2.0, p = 0.008) scores were significantly increased, but BAI scores (4.7±6.5 vs 1.8±2.2, p = 0.07) showed increasing trend in OSA over controls. Significant positive correlations appeared between SaO_2 nadir and MoCA scores (r = 0.56, p = 0.008), and negative correlations emerged between AHI and MoCA scores (r= -0.47, p = 0.03). However, no significant correlations appeared between BDI-II and BAI scores and AHI and SaO_2 nadir values. Early-diagnosed, treatment-naïve OSA subjects show significantly reduced cognitive function and enhanced mood symptoms, and significant associations between cognitive scores and disease severity. The findings of significant relations between cognitive scores and disease severity indicate that higher AHI or lower O_2 saturation may contribute significantly to cognition in the condition.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

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Program#/Poster#: 447.05/EEE8

Topic: F.07. Autonomic Regulation

Support: Global Ischemia Foundation

Department of Molecular and Integrative Physiology

Title: Sympathetic blockade markedly prolongs EEG coherence and delays the onset of cardiac arrest after asphyxia

Authors: *F. TIAN[1], T. LIU[1], G. XU[1], D. LI[1], T. GHAZI[1], T. SHICK[1], A. SAJJAD[1], M. WANG[1,2], P. FARREHI[1], J. BORJIGIN[1];
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Abstract: Sudden cardiac arrest is a leading cause of death around the world. Currently, there are no pharmacological therapies that are effective in preventing sudden cardiac death. Previous studies suggest that overactivation of the sympathetic nervous system plays an important role in mediating sudden cardiac death. Surgical disconnection of the sympathetic efferent signaling via spinal cord transection at cervical level 7 (C7X) significantly extends the survival of both the brain and the heart. The objective of this study is to test the effectiveness of two sympathetic blockers, atenolol and phentolamine, in preventing sudden cardiac death in CO2-mediated asphyxic cardiac arrest model. Rats were divided into 4 groups, which received either atenolol plus phentolamine (10mg/kg, 10mg/kg, n=11), atenolol alone (10mg/kg, n=8), phentolamine alone (10mg/kg, n=7), or saline (n=10), 30 minutes before the onset of asphyxia. EEG (from 6 cortical loci) and EKG signals were simultaneously collected from each rat during the entire process. We found significant increase in EEG coherence duration and EKG signal duration in rats received drugs. Remarkably, rats injected with atenolol plus phentolamine had the most significant increase in EEG coherence duration and EKG signal duration. These results demonstrate that simultaneous blockade of alpha- and beta-adrenergic signaling significantly prolonged the detectable activities of both the brain and the heart during asphyxic cardiac arrest and may offer an effective approach for preventing sudden cardiac death and extend the survival of patients.

Disclosures: **F. Tian:** None. **T. Liu:** None. **G. Xu:** None. **D. Li:** None. **T. Ghazi:** None. **T. Shick:** None. **A. Sajjad:** None. **M. Wang:** None. **P. Farrehi:** None. **J. Borjigin:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.06/EEE9

Topic: F.07. Autonomic Regulation

Title: Alteration of cardiac autonomic function in patients with newly diagnosed epilepsy

Authors: *R. K. GOIT;
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Abstract: Objective: The aim of the study was to determine if heart rate variability (HRV) showed any changes in patients with newly diagnosed epilepsy in comparison with controls. **Methods:** Adult male patients, aged 30-50 years, who had never previously received treatment with antiepileptic drugs were eligible for inclusion in this study. Resting electrocardiogram (ECG) at spontaneous respiration was recorded for 5 min in supine position. Time domain analysis, frequency domain analysis and Poincare plot of HRV were recorded from ECG. **Results:** In time domain measures, the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) and percentage of consecutive RR intervals that differ by more than 50 ms (pNN50) were significantly less in patients with epilepsy. In frequency domain measures, high frequency [(HF) ms^2], HF (nu) and low frequency [LF (ms^2)] were significantly less in patients with epilepsy while LF (nu) and LF/HF were significantly high in patients with epilepsy. In Poincare plot, standard deviation perpendicular to line of Poincare plot (SD1) and standard deviation along the line of entity in Poincare plot (SD2) were significantly less in patients with epilepsy. **Conclusion:** These data suggest that epilepsy patients have an impact on the cardiac autonomic function as measured by HRV.

Disclosures: R.K. Goit: None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

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Program#/Poster#: 447.07/EEE10

Topic: F.07. Autonomic Regulation

Support: NIH Grant HL098351

NIH Grant HL096571

NIH Grant DA08259

Title: Sex and hormonal status influence the redistribution of the AMPA GluA1 receptor subunit in estrogen receptor beta containing paraventricular hypothalamic neurons following slow-pressor angiotensin II hypertension

Authors: *A. C. OVALLES[1], J. MARQUES-LOPES[1], T. A. VAN KEMPEN[1], M. J. GLASS[1], C. IADECOLA[1], E. M. WATERS[2], T. A. MILNER[1,2];

[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [2]Harold and Margaret Milliken Hatch Lab. of Neuroendocrinology, The Rockefeller Univ., New York, NY

Abstract: Hypertension incidence is greater in men than young women, but this relationship reverses as women transition to menopause. A sex-dependent susceptibility to hypertension is recapitulated in rodents following "slow pressor" angiotensin II (AngII) infusion: blood pressure increases in young male, but not young female mice. Slow pressor AngII in the accelerated ovarian failure (AOF) mouse model, which mimics human menopause including the peri-menopause transition, increases blood pressure in peri- and post-AOF mice (Marques-Lopes et al., NEN 2016). Sex-differences in AngII-induced hypertension were reflected by a sex-dependent redistribution of GluN1 to the plasma membrane in estrogen receptor β (ERβ) hypothalamic paraventricular (PVN) neurons. GluN1, a NMDA receptor subunit, is activated by glutamate and leads to recruitment of the AMPA receptor. AMPA, like NMDA, is involved in hypertension regulatory mechanisms. This experiment analyzed the redistribution of GluA1, an AMPA receptor subunit, in ERβ PVN neurons following slow pressor AngII-induced hypertension in males and AOF mice. AOF was induced by injections of 4-vinylcyclohexene diepoxide (VCD; 130mg/kg i.p.) for 15 days in 55 day-old ERβ-enhanced green fluorescent protein (ERβ-EGFP) reporter female mice. Four groups of mice: 1) young (2 mo old) female mice, 2) peri-AOF mice (58 days post-VCD), 3) post-AOF mice (129 post-VCD); and 4) young (2 mo old) male mice were implanted for 14 days with osmotic minipumps containing AngII (600ng/kg/min) or saline, and blood pressure was recorded by tail-cuff plethysmography. Mouse brains were fixed by aldehyde perfusion and PVN sections were labeled for GFP with immunoperoxidase and GluA1 with silver-intensified gold (SIG) and examined by electron microscopy. AngII infusion increased blood pressure in males, peri-, and post-AOF females but not young females. At baseline (i.e., saline), males had significantly more total and cytoplasmic GluA1-SIGs in ERβ-GFP dendrites when compared to all female groups. After AngII infusion, GluA1-SIG distribution in ERβ-GFP dendrites did not change in males. In contrast, the pattern of redistribution of GluA1 SIGs in the females did not reflect the changes in blood pressure after AngII infusion: the total, cytoplasmic and plasmalemmal density of GluA1 SIGs increased in young and post-AOF females but decreased in peri-AOF mice after AngII. The pattern of GluA1 redistribution contrasts that of GluN1 suggesting different roles for each receptor subunit in response to hypertension. In addition, these results support a growing body of evidence that sex dependent mechanisms are in place to influence hypertension.

Disclosures: A.C. Ovalles: None. **J. Marques-Lopes:** None. **T.A. Van Kempen:** None. **M.J. Glass:** None. **C. Iadecola:** None. **E.M. Waters:** None. **T.A. Milner:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.08/EEE11

Topic: F.07. Autonomic Regulation

Support: Canadian Institutes for Health Research (CIHR)

Northern Ontario School of Medicine Faculty Association (NOSM/NOSMFA) Research Development Fund

Title: Renalase expression and regulation in PC12 cells

Authors: *C. R. WILLIAMSON[1], S. KHURANA[4], T. C. TAI[4,1,2,3];
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[4]Med. Sci. Div., Northern Ontario Sch. of Med., Sudbury, ON, Canada

Abstract: Increases in catecholamine (CA) synthesis and secretion by chromaffin cells are often associated with hypertension. The CAs adrenaline, noradrenaline, and dopamine are important regulators of blood pressure through their interaction with adrenergic and dopaminergic receptors. Recently, renalase has been regarded as a secretory protein that may protect against increased levels of circulating CAs. However, the molecular, cellular and physiological mechanisms explaining the protective effects of renalase are poorly understood. The adrenal gland is a major site for CA biosynthesis, however, the regulatory role of renalase in this tissue has not been previously investigated. The purpose of this study is to examine the regulation of renalase in adrenal pheochromocytoma-derived PC12 cells and further to determine its role in CA biosynthesis. The regulation of renalase will also be compared to known regulators of CA biosynthesis. It is hypothesized that renalase secretion is part of a feedback mechanism regulating CA biosynthesis in chromaffin cells. Therefore, renalase may be upregulated when CA biosynthesis-triggering signals are activated. To examine this, PC12 cells were treated with phorbol 12-myristate 13-acetate (PMA), forskolin (Fsk), dexamethasone (Dex), cobalt chloride ($CoCl_2$), adrenaline, or nicotine in a time and dose-dependent manner. Following treatments, standard RT-PCR protocols were used to quantify mRNA for renalase. Preliminary data suggests that PMA, Dex, or Fsk did not influence the expression of renalase at the concentrations and time points examined. In contrast, EGR-1 mRNA was upregulated after 1 hour with 80nM PMA (1.5-fold; $p < 0.01$), and 6 hours with 10μM Fsk (1.4-fold; $p < 0.001$); PNMT mRNA was upregulated at 6 hours and 12 hours with 1μM Dex (>8-fold; $p < 0.001$). These results present the novel finding that renalase is expressed in PC12 cells. However, its regulation in chromaffin cells is not altered by the PKC activation by PMA, PKA activation by Fsk, or cortisol signaling stimulated by Dex. Overall, this study aims to provide a first step in understanding if renalase has a role in the regulation of CA biosynthesis.

Disclosures: **C.R. Williamson:** None. **S. Khurana:** None. **T.C. Tai:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

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Program#/Poster#: 447.09/EEE12

Topic: F.07. Autonomic Regulation

Support: FAPESP (2013/05189-4)

CNPq

PROPE/FUNDUNESP

Title: Chronic inhibition of catalase attenuates the overexpression of AT1 receptor and proinflammatory cytokine mRNA in the hypothalamus of hypertensive rats.

Authors: ***M. R. LAUAR**, D. S. A. COLOMBARI, L. A. DE LUCA JR., P. M. DE PAULA, E. COLOMBARI, C. A. F. ANDRADE, J. V. MENANI;
Dept Physiol. and Pathol., UNESP, Araraquara, Brazil

Abstract: Increased expression of AT1 receptor and proinflammatory cytokines like tumoral necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are present in the central areas involved in cardiovascular control in hypertensive animals. In rats with 2-kidneys, 1-clip (2K1C) hypertension, chronic subcutaneous (sc) administration of the catalase inhibitor 3-amino-1,2,4-triazol (ATZ) reduces mean arterial pressure (MAP) and improves autonomic modulation. In the present study, we analyzed if chronic sc administration of ATZ modifies mRNA expression of AT1 receptors and proinflammatory cytokines in the hypothalamus in 2K1C hypertensive rats. Male Holtzman rats (initial weight 150-180 g, n=7/group) received a silver clip around the left renal artery to generate 2K1C hypertension. Six weeks after the surgery, rats received ATZ or saline sc for 7 days before MAP recording and hypothalamic AT1 receptor, TNF-α and IL-1β mRNA analysis. Baseline MAP increased in 2K1C rats compared to sham (209 ± 4, vs. 99 ± 2 mmHg, respectively). ATZ (600 mg/kg/day) reduced MAP (162 ± 10 mmHg) and the mRNA expression of AT1 receptors (0.74 ± 0.04, vs. saline 1.2 ± 0.2 fold change), TNF-α (0.87 ± 0.1, vs. saline 8.0 ± 1.9 fold change) and IL-1β (0.81 ± 0.11, vs. saline: 1.8 ± 0.75 fold change) in 2K1C hypertensive rats. The results suggest that the anti-hypertensive effects of ATZ are associated with reduction of AT1 receptor, TNF-α and IL-1β mRNA overexpression in the hypothalamus.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.10/EEE13

Topic: F.07. Autonomic Regulation

Title: Pharmacological evidence that NaHS inhibits the vasopressor responses induced by stimulation of the preganglionic sympathetic outflow in pithed rats

Authors: ***A. SACHEZ-LOPEZ**, S. HUERTA-DE LA CRUZ, E. J. GUTIÉRREZ-LARA, J. H. BELTRAN-ORNELAS, D. CENTURIÓN;
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Abstract: It has been reported that i.v. administration of NaHS, a donor of H2S, elicited dose-dependent hypotension although the mechanisms are not completely understood. In this regard, several mechanisms could be involved including the inhibition of the vasopressor sympathetic outflow. Thus, this study was designed to determine the potential capability of NaHS to mediate inhibition of the vasopressor responses induced by preganglionic sympathetic stimulation. For this purpose, Wistar rats were anaesthetised, pithed and cannulated for drug administration. In animals pre-treated with gallamine, the effect of i.v. infusion of NaHS (310 and 560 μg Kg^{-1} min^{-1}) or its vehicle (phosphate buffer) was determined on the vasopressor responses induced by: (1) sympathetic stimulation (0.03-10 Hz); (2) i.v. bolus injections of exogenous noradrenaline (0.03-3 μg Kg^{-1}); or (3) methoxamine (1-100 μg Kg^{-1}). The vasopressor responses induced by preganglionic sympathetic stimulation were dramatically and dose-dependently inhibited by i.v. infusion of NaHS (310 and 560 μg Kg^{-1} min^{-1}), but not by vehicle, particularly at high frequencies. In marked contrast, the vasopressor responses to exogenous noradrenaline or methoxamine were not inhibited by the above doses of NaHS or its vehicle. The above results, taken together, demonstrate that NaHS inhibited the vasopressor responses induced by preganglionic sympathetic outflow by a prejunctional mechanism. This is the first evidence demonstrating this effect by NaHS and may contribute, at least in part, to the hypotension induced by NaHS.

Disclosures: **A. Sachez-Lopez:** None. **S. Huerta-De la Cruz:** None. **E.J. Gutiérrez-Lara:** None. **J.H. Beltran-Ornelas:** None. **D. Centurión:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.11/EEE14

Topic: F.07. Autonomic Regulation

Title: Intraneural interrogation of vagus nerve activity

Authors: A. KANNEGANTI, A. S. DESHMUKH, *M. I. ROMERO-ORTEGA;
Bioengineering, Univ. of Texas at Dallas, Richardson, TX

Abstract: Somatic peripheral nerve intraneural multielectrode interfaces have been extensively used to decode and encode underlying neural activity towards neuromodulation and advanced bionic systems applications. However, the intraneural activity of autonomic nerves is not well understood, despite of their clinical relevance as exemplified by vagus nerve stimulation, which is approved for the treatment of various pathological conditions including stroke, obesity, epilepsy, and hypertension. A limiting factor in using intraneural electrodes in the rat autonomic nerves is their small size. Here we use indwelling microelectrodes to evaluate the vagus nerve activity in response to inflammatory cytokines. This is important as this cholinergic anti-inflammatory pathway plays a key role in maintain homeostasis via neuro-endocrine-immune axis. Slanted microelectrode arrays were implanted into the left cervical vagus nerve of adult female Lewis rats. Intraneural recordings were done in acute anesthetized animals (N=6). Omniplex neural data acquisition system (Plexon Inc.,) was used to record the single unit and multi-unit activity, and offline filtering followed by PCA (Principal component Analysis) based spike sorting, was used to identify individual spikes. Multiple distinct biphasic single unit spikes were observed with peak-to-peak voltages ranging between 80-150μV. At the end of each study session, lidocaine was applied topically and suppression of the spikes was used to confirm the neuronal activity. Multiple spikes identified across animals showed a repetitive burst-firing pattern that temporally correlated with breathing. Interrogation of the modulation in the inherent vagal activity in response to systemic and local delivery of inflammatory cytokines TNFα, IL-6 and Il-4 in rodent animal model is currently under investigation. Preliminary results show an observable change in firing rate in response to cytokine administration. In summary, we report on the use of indwelling microelectrode arrays to interrogate the inherent neuronal activity in the vagus nerve and use it to evaluate their activity in relation to respiration and inflammation

Disclosures: A. Kanneganti: None. **A.S. Deshmukh:** None. **M.I. Romero-Ortega:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.12/FFF1

Topic: F.07. Autonomic Regulation

Title: Combined administration of insulin and leptin significantly increased Fos production in the arcuate nucleus and renal sympathetic nerve activity

Authors: H. HABEEBALLAH, N. ALSUHAYMI, M. STEBBING, T. JENKINS, *E. A. BADOER;
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Abstract: Leptin has sympatho-excitatory effects on renal sympathetic nerve activity (RSNA). Insulin also induces increases in RSNA. The aim of this study was to investigate the effects of the intracerebroventricular (ICV) administration of insulin and leptin together on RSNA, mean arterial pressure (MAP), heart rate (HR) and distribution of Fos protein in autonomic brain nuclei. Anaesthesia in male Sprague-Dawley rats was induced with isoflurane (2-5%) in O_2 and maintained with urethane (1.4-1.6 g/kg iv). RSNA, MAP, and HR were recorded before and for 180 minutes after the ICV injection of saline (control, n=5), leptin (7 µg, n=5), insulin (500 mU, n=4), and the combination of both insulin and leptin (leptin was administered 15 minutes after insulin, n=4). At the end of each experiment the animals were perfused and the brains processed immunohistochemically to detect Fos, a protein marker of increased neuronal activation. RSNA was increased significantly more when leptin and insulin were administered together compared to either leptin or insulin alone. Insulin alone significantly increased HR by (47±11 b/min), however, the presence of leptin prevented that HR response. No change in MAP was observed with any treatment. Fos production was examined in the following brain nuclei; arcuate nucleus (ARC), hypothalamic paraventricular, supraoptic, lamina terminalis and in autonomic nuclei in the medulla oblongata. Only in the arcuate nucleus did ICV injection of leptin and insulin together increase the number of Fos-positive cell nuclei significantly more than the increases observed following leptin or insulin alone. The results suggest that in conditions where leptin and insulin are elevated, such as obesity, the enhanced effects on RSNA may be contributing to the elevation of RSNA seen in such conditions and this may contribute to the cardiovascular complications. Further, the results suggest that the arcuate nucleus is an important common site of cardiovascular integration where both leptin and insulin interact

Disclosures: H. Habeeballah: None. **N. Alsuhaymi:** None. **M. Stebbing:** None. **T. Jenkins:** None. **E.A. Badoer:** None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

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Topic: F.07. Autonomic Regulation

Support: NIH Grant 5R01HL028785

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NHLBI F32 HL127975

Title: Cardiovascular control by neurons of the rostral ventrolateral medulla in conscious rats

Authors: *I. C. WENKER[1], C. ABE[2], R. L. STORNETTA[1], P. G. GUYENET[1];
[1]Pharmacol., Univ. of Virginia, Charlottesville, VA; [2]Gifu Univ., Gifu, Japan

Abstract: The rostral ventrolateral medulla (RVLM) contains excitatory neurons that are believed to be critical for blood pressure (BP) control. In anesthetized animals, these baro-regulated neurons are extremely active and essential to maintain BP, but their contribution in conscious animals is less clear. In conscious rats, optogenetic activation of an RVLM subpopulation (the C1 neurons) increases BP and sympathetic nerve activity (SNA), but selective destruction of these neurons alters BP very little. This lack of effect could be explained by adaptive changes or by the fact that the C1 neurons contribute little to BP at rest in the conscious state. Thus, we decided to measure the contribution of the RVLM neurons to BP maintenance in intact conscious animals using transient and reversible inhibition. To do this, RVLM neurons were bilaterally transduced to express the light-driven proton pump Archaerhodopsin (ArchT) by viral vector injection and optical fibers were surgically implanted to deliver green laser light (530 nm, 5-7 mW) to the RVLM. Expression of ArchT was driven by either CamKIIa, a pan-excitatory neuron promoter, or PRSx8, a synthetic promotor specific to the C1 neuronal population, resulting in two experimental rat populations; CamK-ArchT (n=5) and C1-ArchT (n=8) rats. Four weeks after viral delivery, telemetric BP probes were implanted. After recovery, rats were placed in a plethysmography chamber on top of a wireless receiver, to record cardiorespiratory parameters. As proof-of-principle, single-unit recordings in 3 anesthetized rats revealed that a majority (7/11) of barosensitive were reversibly inhibited (82.6 ± 11.6 % reduction, $p = 0.01$). In both CamK-ArchT and C1-ArchT conscious rats under normoxic conditions (21% FiO_2), bilateral inhibition for 10 s had virtually no effect on BP. However, the ArchT-induced hypotension increased significantly during hypoxia (Cam-ArchT: -13.1 ± 1.2 mmHg in 12% O_2 versus -5.2 ± 0.8 in 21% O_2, $p = 0.0006$, C1-ArchT: -20.9 ± 3.2 mmHg in 12% O_2 versus -3.4 ± 1.0 in 21% O_2, $p = 0.0001$), but not during hypercapnia (Cam-ArchT: -7.2 ± 0.7 mmHg in 6% CO_2 vs. -5.2 ± 0.8 in 0% CO_2, $p = 0.080$, C1-ArchT: -6.4 ± 1.0 mmHg in 6%

CO_2 vs. -3.4 ± 1.0 in 0% CO_2, p = 0.207). Even though Cam-ArchT and C1-ArchT rats exhibited opposite respiratory effects due to ArchT inhibition, similar cardiovascular effects were observed, indicating the pressor response was not due altered respiratory activity. In conclusion, whether targeting all RVLM excitatory neurons or C1 neurons specifically, RVLM neurons seem to be relatively inactive in normoxic unstressed rats, but become activated during hypoxia to prevent BP from falling, presumably by increasing SNA.

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Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 447.14/FFF3

Topic: F.07. Autonomic Regulation

Support: Heart and Stroke Fdn. of Ontario

Title: Ventrolateral medullary pathways mediate cardiovascular responses to activation of the ventral tegmental area.

Authors: *J. CIRIELLO;
Univ. Western Ontario, London, ON, Canada

Abstract: Activation of the ventral tegmental area (VTA) elicits sympathoinhibitory responses. However, the brainstem pathways mediating these effects on the cardiovascular system are unknown. In this study, experiments were done in alpha-chloralose anaesthetized, paralysed and artificially ventilated rats to investigate the medullary pathways mediating the mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve (RSN) responses elicited by l-glutamate (Glu) stimulation of VTA neurons. In the first series, microinjection of Glu at depressor sites was found to induce c-fos expression within the caudal ventrolateral medulla (CVLM), bilaterally. In contrast, little c-fos expression was observed within other medullary areas, including the rostral ventrolateral medulla (RVLM). In the second series, to investigate whether CVLM mediated the VTA depressor responses, bilateral injections (100 nL) of the synaptic blocker cobalt chloride ($CoCl_2$; 5 mM) were made into CVLM. The $CoCl_2$ injections significantly attenuated the MAP (~ 82 %), HR (~ 74 %) and RSN (~87%) responses elicited by stimulation of VTA. Control injections of the vehicle into CVLM or injections of $CoCl_2$ into regions immediately dorsal to the CVLM region, bilaterally, did not alter the magnitude of the cardiovascular responses to VTA stimulation. On the other hand, bilateral injections of $CoCl_2$ into the rostral ventrolateral medulla (RVLM) similarly attenuated the VTA responses. In

addition, to determine whether gamma aminobutyric acid (GABA) and/or glutamate containing systems were involved in mediating these effects through the CVLM, bilateral injections of the $GABA_A$ antagonist bicuculline methiodide (Bic; 0.4 mM) or the non-specific glutamate receptor antagonist kynurenic acid (KYN 0.15 M) were made into CVLM or RVLM. RVLM injections of Bic significantly attenuated the MAP**,** HR and RSN responses to VTA stimulation, whereas, bilateral injections of Bic into CVLM did not alter the VTA responses. Bilateral injections of the $GABA_B$ antagonist phaclofen or baclofen (5 mM) into the same CVLM or RVLM regions did not alter the cardiovascular responses to stimulation of the VTA. Finally, bilateral injections of KYN into the CVLM significantly attenuated the MAP, HR and RSN responses, whereas KYN injections into RVLM had no effect on the cardiovascular responses to VTA stimulation. Taken together, these data suggest that VTA activates a descending glutamatergic pathway that in turn activates GABAergic neurons in CVLM that inhibit RVLM sympathoexcitatory neurons that control the vasculature and heart through the activation of $GABA_A$ receptor mechanisms. This work was supported in part by HSFO.

Disclosures: J. Ciriello: None.

Poster

447. Autonomic Control: Cardiovascular Regulation II

Location: Halls B-H

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Support: Taiwan NSC 94-2320-B-303-003

Taiwan NSC 95-2745-B-320-004-URD

Taiwan MOST 103-2320-B-303-001-MY3

Title: Ketamine and amphetamines inhibit neurogenic nitrergic vasodilation of porcine isolated basilar arteries

Authors: M.-F. CHEN[1,2], S.-Y. LAI[3], P.-C. KUNG[3], Y.-C. LIN[3], H.-I. YANG[1], P.-Y. CHEN[1], *T. J.-F. LEE[4,1,5];
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Abstract: Chronic abuse of ketamine and amphetamines is associated with incidence of hypertension and strokes involving activation of sympathetic activities. Large cerebral arteries at the base of the brain from several species receive dense sympathetic innervation which upon activation causes predominant parasympathetic-nitrergic vasodilation with increased regional blood flow via axo-axonal interaction mechanism. The present study was designed to examine effects of ketamine and amphetamine analogs on axo-axonal interaction-mediated neurogenic nitrergic vasodilation in porcine basilar arteries using techniques of blood-vessel myography, patch clamp and two-electrode voltage clamp, and calcium imaging. In U-46619-contracted basilar arterial rings, nicotine (100 μM) and transmural nerve stimulation (TNS, 8 Hz) elicited neurogenic nitrergic vasodilations. Ketamine and amphetamine analogs concentration-dependently inhibited nicotine-induced nitrergic vasodilation without affecting that induced by TNS. Ketamine and amphetamine analogs also concentration-dependently blocked nicotine-induced inward currents in Xenopus oocytes expressing alpha3beta2-nicotinic acetylcholine receptors (nAChRs), and nicotine-induced inward currents and calcium influxes in rat superior cervical ganglion neurons. The potency in inhibiting both inward-currents and calcium influxes is comparable among the analogs. These results indicate that ketamine and amphetamine analogs, by blocking nAChRs located on cerebral perivascular sympathetic nerves, reduce nicotine-induced, axo-axonal interaction-mediated neurogenic dilation of the basilar arteries. Chronic abuse of these drugs may interfere with normal sympathetic-parasympathetic interaction mechanism resulting in diminished neurogenic vasodilation (supported by MOST and Tzu Chi Foundation).

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Poster

447. Autonomic Control: Cardiovascular Regulation II

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Program#/Poster#: 447.16/FFF5

Topic: I.07. Data Analysis and Statistics

Support: University of Zurich 320030_1449586/1

Wellcome Trust 091593/Z/10/Z

Title: Assessing fear memory by modelling conditioned bradycardia and respiratory responses

Authors: ***G. CASTEGNETTI**[1], A. TZOVARA[1], M. STAIB[1], P. C. PAULUS[2], N. HOFER[1], D. R. BACH[1,3];

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Abstract: Across species, cued fear conditioning is a common experimental paradigm to investigate aversive Pavlovian learning. While fear-conditioned stimuli (CS+) elicit overt behaviour in many mammals, this is not the case in humans. Instead, autonomic nervous system activity is used to quantify fear memory in humans, typically measured by skin conductance responses (SCR). Here, we investigate whether heart period responses (HPR) and respiratory amplitude responses (RAR), evoked by the CS+, are suitable as measures of fear memory in humans. Heart beats are identified from electrocardiogram or pulse oximetry, and RAR from a single-belt chest bellows system. Peak-scoring reveals fear-conditioned heart rate deceleration (bradycardia) and distinguishes CS+ from non-reinforced stimuli (CS-) in each of four datasets. RAR differ between CS+/CS- across conditions only in one out of three analysed datasets. We then develop a Psychophysiological Model (PsPM) for fear-conditioned responses in each modality. These PsPMs are inverted to yield estimates of autonomic input into the cardiac or the respiratory system. We show that the sensitivity to distinguish CS+ and CS- (predictive validity) is higher for model-based estimates than peak-scoring analysis, both for HPR and RAR. Model-based estimates of RAR significantly discriminate CS+/CS- in all three analysed datasets. Finally, we compare the predictive validity of the best performing model-based implementation of these modalities to SCR. We show that HPR significantly outperforms both SCR and RAR, which in turn perform similarly. Our work provides two novel tools to investigate fear memory in humans and potentially allows direct comparison between species.

Disclosures: G. Castegnetti: None. **A. Tzovara:** None. **M. Staib:** None. **P.C. Paulus:** None. **N. Hofer:** None. **D.R. Bach:** None.

Poster

448. Gastrointestinal: Reproductive Regulation

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Topic: F.07. Autonomic Regulation

Support: R01 DK57284

R37 DK54824

Rising Star Urology Research Award

Title: Chronic stress is associated with augmented urinary bladder neuronal sprouting

Authors: *B. M. MCDONNELL[1], A. KULLMANN[1], A. WOLF-JOHNSON[1], A. KANAI[1], L. RODRIGUEZ[2], L. BIRDER[1];
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Abstract: *Background:* A hallmark of functional pain syndromes such as interstitial cystitis/bladder pain syndrome (IC/BPS) is pain in the absence of other demonstrable pathology. There is ample evidence that acute stress increases bladder pain and urgency in these individuals. Recent findings support increased bladder hyperalgesia and urinary frequency in rats exposed to chronic (10 day) water avoidance stress (WAS). Though underlying mechanisms have not been fully explored, studies have shown that nerve growth factor (NGF) plays an important role in hyperalgesic responses in both somatic and visceral structures. Further, stress is known to affect the expression levels of neurotrophic factors. In addition, over-expressing NGF has also been shown to result in neural sprouting which may be involved in mechanisms underlying hyperalgesia and pain.
Purpose: In order to understand how changes in peripheral neural innervation may contribute to bladder hyperalgesia and pain in this model, we examined changes in sensory and autonomic innervation of the bladder.
Methods: Adult female Wistar-Kyoto rats (12 wks age, 200-250 gm) were placed on a pedestal in a water water-filled container. All procedures were conducted with approval of University of Pittsburgh Institutional Animal Care and Use Committee. Animals were exposed to WAS (or handled controls) one hr/day x10 days between hours 8 AM-12 PM to minimize circadian effects. To investigate the density and distribution of fibers in bladder we used immunocytochemistry to process bladder cross cryosections (20uM; postfixed following bladder removal from deeply anesthetized rats prior to sacrifice; n=3 rats), specifically against calcitonin gene related peptide (CGRP; sensory fibers); growth associated protein 43 (sprouted nerve fibers) and tyrosine hydroxylase (TH; sympathetic fibers) and DAPI to label nuclei.
Results: Our findings reveal a significant increase (approx. 2-fold) in CGRP staining in WAS bladders (vs. control) with the greatest density within the bladder mucosa. We also find augmented GAP43 (approx. 2-fold) staining as well as TH (approx. 1.5-fold) staining in WAS bladders (vs. control), the latter surrounding the vasculature. The increase in neural sprouting following chronic WAS also correlated with elevated NGF bladder expression.
Conclusions: Alterations in both sensory and autonomic neural innervation in the bladder may represent an important mechanism that contributes to hypersensitivity/urgency in IC/BPS models of bladder pain.
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Poster

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Title: Electrical stimulation to increase colonic activity

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Abstract: Neurogenic bowel dysfunction is a common condition for persons with spinal cord injury that significantly disrupts daily bowel management and impairs quality of life. We aim to develop an approach using functional electrical stimulation to restore bowel function for persons with spinal cord injury. The objectives of this pilot study were to determine if reflex colonic activity can be elicited via electrical stimulation of the colon and to identify the effect of stimulation variables, such as stimulation pattern and electrode location. Experiments were conducted in seven neurally intact cats under chloralose anesthesia to maintain reflexive activity. Proximal colon, distal colon, and rectal pressures were recorded via balloon catheters. Proximal colon, distal colon, and rectum were stimulated with continuous and burst patterns. Constant frequency, surface colon stimulation (20-40 Hz, 30 s) evoked localized colon contractions in the colon segment directly below the electrodes in all cats. Burst pattern stimulation (5-15 pulses at 100 Hz every second) also evoked colonic responses in 3/3 animals. 20 Hz stimulation produced ischemia and a tetanic colon contraction. Burst pattern stimulation did not alter tissue appearance, and colonic pressures increased more slowly and included a slow ripple. Pressures increased with increasing stimulus amplitude, frequency, pulse width, and burst number. Rectal stimulation did not evoke significant responses. Proximal colon surface stimulation resulted in only proximal colon pressure increases. Distal colon surface stimulation generated both distal and proximal colon pressure increases. Isoflurane anesthesia eliminated proximal pressures and reduced distal pressures, suggesting that reflex pathways were activated via distal colon stimulation. Colonic pressures can be produced via both direct and reflex pathways using electrical colon stimulation. A neural stimulation approach has the potential to restore colonic activity.

Disclosures: **D.J. Bourbeau:** None. **K. Aamoth:** None. **K. Gustafson:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: P20 DK-103086

Title: Identifying brain networks controlling micturition and continence in mouse

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Abstract: Lower urinary tract symptoms (LUTS) are extremely common and enormously debilitating. A significant component of LUTS is due to failure of nervous control of bladder function, or failure of neural pathways to compensate for bladder dysfunction. However, it is not clearly understood how the brain controls bladder filling and voiding or how the micturition reflex is inhibited if the timing for voiding is not right. Prior studies have shown that the pontine micturition center (PMC, aka Barrington's nucleus) directly controls voiding. Within the PMC, neurons expressing corticotropin releasing hormone (PMC^{CRH}) project axons directly to sacral spinal cord nuclei that control bladder contraction and sphincter relaxation. Here we show that PMC^{CRH} neurons are critical for voiding, and identify a network of afferent neurons, across several forebrain and brainstem regions, which directly modulate PMC^{CRH} and affect voiding behavior. First, stimulating PMC^{CRH} neurons using Gq DREADDs produces urinary frequency in awake-behaving mice and on the anesthetized cystometrogram (CMG). Conversely, selectively ablating these neurons using diphtheria toxin A leads to urinary retention and eliminates the CMG voiding reflex. Next, to identify input connections to the PMC, and specifically those controlling PMC^{CRH} neurons, we used CTb and modified rabies. To confirm which retrogradely labeled neurons target PMC^{CRH} neurons specifically, we performed viral anterograde tracing. Afferents to PMC^{CRH}neurons are located in PAGvl, the preoptic area, the lateral hypothalamic area, and other sites. We confirmed monosynaptic connectivity using electrophysiological recordings for select sites. Then, to test the functional consequence of afferent neuron activity, we manipulated the activity of these clusters of neurons or by stimulating their axon terminals within the PMC. We used a novel non-invasive assay for mouse voiding: micturition video

thermography (MVT), to track voiding behavior in these awake-behaving mice. In select cases we combine MVT with telemetric measurement of bladder pressure or urethral sphincter EMG. After MVT we repeat these manipulations during an anesthetized CMG. Taken together, we have identified a network of neurons that can control urinary voiding and continence through PMC^{CRH} neurons. Next, we will determine the necessity of select afferent sites for voiding, and the effects of removing their modulatory input for continence. This information will help us understand how forebrain, brainstem and spinal inputs converge to control bladder filling and voiding, and will allow more detailed studies of the neurologic mechanisms of LUTS in mice and humans.

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Poster

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Topic: F.07. Autonomic Regulation

Title: Impact of decentralization on cholinergic transmission and neuronal excitability in mouse major pelvic ganglia

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Abstract: Damage to the pelvic nerve due to complications from surgeries carried out in the pelvic area often results in micturition and erectile dysfunctions. We are interested in the impact of loss of input due to damage to the nerves innervating the major pelvic ganglia (MPG) on the excitability, firing properties, and cholinergic neurotransmission of the post-ganglionic neurons of the MPG. The MPGs of mice contain postganglionic neurons innervating urinary bladder, the genitals and distal colon. Pelvic ganglia primarily receive cholinergic parasympathetic inputs from the preganglionic neurons in the sacral cord through pelvic nerve, with a smaller number of sympathetic neurons receiving inputs from the lumbosacral cord through the hypogastric nerve. The axons from the pelvic ganglion synapse onto postganglionic neurons on the target organ, releasing acetylcholine (ACh - parasympathetic) and noradrenaline (sympathetic) to impact target function and activity. We studied the effects of loss of input to postganglionic MPG neurons via unilateral transection of both pelvic and hypogastric nerves in adult mice. We employed molecular and electrophysiology techniques to examine ipsilateral decentralized MPGs, contralateral intact MPGs, and sham-operated adult Swiss Webster mice of both sexes.

To determine impacts of decentralization on synaptic strength between pre- and post-ganglion neurons of the MPG, we measured excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs) in response to local application of ACh. We further characterized the major subunits involved in the cholinergic transmission by measuring ACh-activated currents before and after application of subunit-specific blockers. To investigate impacts of injury on excitability of the MPG neurons, we measured passive properties of these neurons, as well as firing patterns in response to DC current injection. We also measured the mRNA abundance of α2, α3, α4, α5, α6, α7, β2 and β4 nicotinic receptor subunits in whole MPGs using quantitative PCR (qPCR). Our preliminary data indicate that after acute decentralization (1-3 days), the mRNA transcript numbers for α3, α7, and β4 were significantly decreased, while the remainder remain unchanged. These results suggest that acute injury results in downregulation of receptor subunits responsible for cholinergic transmission in the MPG, and may have impacts for long-term recovery of bladder function following pelvic nerve injury.

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Poster

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Support: NIH Grant RO1DK084321

Title: Sensory innervation of the pancreatic islet

Authors: *M. MAKHMUTOVA, R. RODRIGUE-DIAZ, J. ALMACA, J. WEITZ, E. BERNAL-MIZRACHI, A. CAICEDO;
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Abstract: The brain receives and processes stimuli from the viscera to adjust autonomic output and regulate glucose metabolism as part of body homeostasis. Visceral stimuli are detected by free nerve endings of the Vagus nerve and are transmitted to the hindbrain via sensory neurons. However, it is still unknown what signals activate vagal sensory neurons in the pancreas and which neural circuits process this information. We are developing tools to characterize the molecular and functional profile of vagal sensory neurons that innervate pancreatic islet. We use *in-vivo* and *in-situ* Ca^{2+} imaging of the nodose ganglion in combination with retrograde tracing from the pancreas to identify specific subpopulations of the vagus nerve that respond to islet-derived substances and to determine the molecular nature of sensory stimuli. In addition, we will

implement pharmacogenetic approach to manipulate islet sensory innervation to study its role in glucose metabolism. We will use adeno-associated viral vectors, that express synthetic G-protein coupled receptor (DREADD-GPCR) that can be activated by physiologically inert molecule (CNO). The virus will be injected into the pancreas or nodose ganglion, and depending on the activatory or inhibitory nature of DREADD-GPCR we will stimulate or inhibit activity of sensory neurons that innervate pancreatic islet. Thus we have a model where we can characterize and manipulate islet-specific neural circuits. Altogether we expect this study to provide insight into pancreatic sensory innervation and its contribution to glucose metabolism.

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Poster

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Title: Anxiogenic effect of probiotics, prebiotics and synbiotics on healthy juvenile rats

Authors: *J. A. BRAVO[1], C. BARRERA-BUGUEÑO[1], J. ESCOBAR-LUNA[1], O. REALINI[1], R. SOTOMAYOR-ZÁRATE[2], M. GOTTELAND[3], M. JULIO-PIEPER[1];
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Abstract: Interventions on the gut microbiota have an impact on healthy animal behavior. Indeed, the use of probiotics and prebiotics has been shown to alter behavior in adult animals and healthy volunteers, but little is known on the effects of such interventions in younger individuals. In order to evaluate this, weaned male Sprague-Dawley rats (post-natal day 21, PND21) were fed with the probiotic *Lactobacillus casei* L-54-2-33 (10^4 cfu's/ml), the prebiotic inulin (16mg/ml), and the mixture of both (synbiotic) for 14 days. All treatments were added to the drinking water, while control rats were given water alone. On PND34, behavior was evaluated in the open field (OF) test, and on PND35 animals were subjected to the elevated plus maze (EPM) test. 30 mins after the EPM rats were decapitated; trunk blood was collected for plasma corticosterone

(CORT) measurements and brains were collected to determine hippocampal expression of 5-HT_{1A} mRNA through *in situ* hybridization.
There was no difference in weight gain between treated and control rats. Behavioral analyses revealed that animals treated with the probiotic, prebiotic and synbiotic had fewer entries to the central area of the OF arena and spent more time in the periphery of the apparatus in comparison to control animals. In the EPM, synbiotic fed rats spent more time in the open arms in comparison to probiotic and prebiotic fed rats. In addition, higher levels of stress-evoked CORT were observed in probiotic fed rats in comparison to control animals, while no increase in plasma CORT was observed in synbiotic fed rats in comparison to controls. Interestingly, basal levels of CORT evaluated in a different cohort that was not exposed to behavioral tests, were higher in prebiotic fed rats in comparison to control animals. Also in this last cohort, densitometric analyses revealed that 5-HT_{1A} mRNA expression was increased in the dentate gyrus and cornus ammonis 1 and 3 of synbiotic fed rats in comparison to all other groups. Together these results suggest that interventions in the microbiota of young healthy animals evoke behavioral changes in response to stressful situations, which seem to be independent of hypothalamus-pituitary-adrenal axis activation. Synbiotic fed animals had higher levels of hippocampal 5-HT_{1A} transcript in comparison to all other groups, and moreover, this group was the only one to have reduced anxiety-like behaviors in the EPM. These latter results suggest that there is a complex interaction between endogenous and exogenous bacteria and prebiotic compounds, having different effects on the microbiota-gut-brain axis. In addition, the animal's age might also influence the behavioral outcome of such interventions.

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Poster

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Topic: F.07. Autonomic Regulation

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Title: Differential effects of SSRIs on rat intestinal permeability and innate immune response markers

Authors: *M. JULIO-PIEPER, J. EYZAGUIRRE-VELASQUEZ, C. GONZALEZ-ARANCIBIA, L. OLAVARRIA-RAMIREZ, J. ESCOBAR-LUNA, C. BARRERA-BUGUEÑO, J. A. BRAVO;
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Abstract: Acute and chronic psychological stress has been associated with increased gut permeability. Antidepressant drugs have been used to ease gastrointestinal symptoms that are frequently comorbid with anxiety and depression. Here, the focus has been to treat gut dysmotility and abdominal pain; however, the effects of antidepressant therapy on gut permeability remain unexplored. Selective serotonin reuptake inhibitors (SSRIs) have anti-inflammatory effects and can inhibit toll-like receptor (TLR) activity. Because inflammatory/immune mediators are key modulators of epithelial permeability, we aimed to investigate whether the SSRIs sertraline and citalopram are able to prevent experimentally-induced intestinal barrier deterioration in the rat.
We have previously used protein malnutrition (4% instead of 26% protein in a control diet) to disrupt rat gut barrier function without elevating plasma corticosterone. After weaning at postnatal day (PND) 21, animals received control diet until PND 39. Thereafter, they were separated into a control group, which received the same diet until PND 60, and a low protein (LP) group, which was fed a low protein diet from PND 40 to 60. Simultaneously, LP rats were received sertraline (30 mg/kg/day, p.o.) or citalopram (20 mg/kg/day, p.o.) from PND 40 to 60. Control and non-treated LP rats received saline daily by oral gavage. Intestinal tissue was collected to evaluate transepithelial electrical resistance (TEER) as a functional measure of gut permeability. Mucosal levels of tight-junction protein occludin and phospho-IRF3, a transcriptional factor linked to TLR-3 activation, were analyzed by western blot.
LP rats displayed reduced TEER, decreased occludin levels and increased phospho-IRF3 levels in ileum and colon. Sertraline significantly prevented TEER reduction in LP rat colon. A similar but non-significant trend was observed in the ileum of sertraline-treated LP rats and both ileum and colon of citalopram-treated LP rats. The decrease in occludin was significantly prevented only by citalopram in the colon of LP animals; however none of the drugs improved occludin levels in the ileum. Regarding phospho-IRF3, sertraline did not prevent its increase in the gut of LP rats; on the other hand, citalopram had a protective effect but only in LP rat ileum.
Our results show that two SSRIs induce distinct and significant effects on gut permeability. This, together with the reduction in phospho-IRF3, a molecule associated to the innate immune response, indicates that barrier protection and anti-inflammatory effects of SSRIs on non-neuronal cells could be an additional mechanism in the relief of gastrointestinal symptoms.

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Poster

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Topic: F.07. Autonomic Regulation

Title: Cisplatin causes up regulation of orexin R-1 receptor and serotonin in nodose ganglion of the least shrew (Cryptotis parva)

Authors: *M. S. AL-TIKRITI, W. KHAMAS, S. CHEBOLU, N. DARMANI;
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Abstract: Previously we have found that cisplatin has significant impact on the enterochromaffin cells of gastrointestinal tract (GIT) and brainstem emetic nuclei function via release of several emetogenic neurotransmitters/mediators including 5-HT, substance P (SP). Vagal afferent neurons residing in the nodose ganglia (NG) is the primary sensory neurons that receive signals arising from the gut lumen and project to the nucleus of solitary tract (NTS) of the brainstem.. This has been shown by immunocytochemical techniques in rat and cat, and is the major afferent projection of the vagus nerve, whose cell bodies are located inside the (NG). The purpose of this study was to determine whether a small vomit competent species, the least shrew, shares a similar biochemical and anatomical profile observed in other species, and whether or not cisplatin extends its effect on the neurons of nodose ganglia. Thus, we used serotonin immunocytochemistry technique to detect serotonergic neurons, and OX-R1antibodies against orexin-1 receptors, since Orexin-A and -B are brain gut peptides that stimulate food intake via Orexin-R1 and -R2 receptors. We used six shrews; three were used as control and the others were injected with cisplatin (10 mg/kg- i.p.) which were sacrificed 24 hours post injection. The whole heads were decalcified with 2% formic acid for 2 weeks after fixation with 10% buffered neutral formalin for 5 days. The samples were processed for paraffin embedded blocks and sectioned at 10 μm thickness. The data shows that the least shrew shares similar findings with the rat, cat and human by having co-localized serotonergic neurons and by expressing OX-R1 in cells of the nodose ganglia. In addition, cisplatin causes severe damage to the ganglionic cells as manifested by shrinkage and pyknotic nuclei of the nodosal neurons. Interestingly, OX-R1 was expressed in three different population of cells; large neurons show less activities, medium size, and glial (satellite) cells express more as shown by immunostaining. The present study provides new information regarding serotonin and OX-R1 receptors in the neurons as well as satellite cells of the nodose ganglia and their role in physiological central effects that is mediated by afferent vagal fibers following cisplatin treatment.

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Poster

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Topic: F.07. Autonomic Regulation

Title: Progressive lower urinary tract dysfunction in mice with alkaline ceramidase 3 deficiency

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Abstract: We have previously demonstrated that alkaline ceramidase 3 (Acer3) activity in the nervous system increases with age, suggesting a critical role in age-related maintenance of sphingolipid homeostasis. Knockout (KO) of Acer3 increases the concentration of long-chain ceramides, especially in older mice, and prevents the age-related increase in sphingosine and S1P concentration in the mouse nervous system. At the same time, Acer3 KO mice develop progressive urine retention with age, which is more pronounced in female mice. Since sphingolipid levels of the nervous system are more impacted than those of the bladder in Acer3 KO mice, we studied how Acer3 affects (1) bladder size to characterize the morphological phenotype and (2) the micturition reflex to assess possible changes in the neural control of the lower urinary tract (LUT). To this end, wild-type (WT), heterozygous (Het), and KO mice received monthly ultrasounds of the bladder to measure changes in bladder size over time. Female WT, Het, and KO mice also underwent cystometric studies at 4 and 6 months of age in order to identify possible early physiological changes that may contribute to LUT dysfunction. The ultrasound data indicate that the bladders of Acer3 KO mice begin to enlarge at around 6 months of age and lack any gross abnormalities, e.g., bladder stones. For cystometry, mice were anesthetized with isoflurane (1.5-2%) for the initial surgical procedure to insert a catheter into the dome of the bladder and then maintained with urethane (1.2-1.5 g/kg) for the duration of the recording. Micturition reflexes were elicited by continuous intravesical infusion of saline into the bladder, and bladder pressure data were recorded for 1.5-2 hours after establishing a steady-state pattern of reflex micturition. Cystometric data indicate that 4-month-old Acer3 KO mice lack strong voiding bladder contractions and exhibit frequent non-voiding bladder contractions. The Acer3 KO mice also are unable to fully evacuate the bladder during continuous slow infusion of saline into the bladder, resulting in residual volume and enlarged bladder. The changes in reflex micturition demonstrated with cystometry occur prior to onset of gross bladder pathology indicated with ultrasound, suggesting that Acer3 deficiency disrupts the neural control of micturition and potentially produces a novel model of age-related underactive bladder syndrome.

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Poster

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Title: Acute intermittent hypoxia-induced long-term facilitation of micturition-related external oblique muscle activity

Authors: M. CATEGE[1], N. P. PHAGU[1], I. C. SOLOMON[2], *W. F. COLLINS, III[1];
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Abstract: Exposure to acute intermittent hypoxia (AIH) produces a sustained increased in respiratory motor output (long-term facilitation (LTF)). This AIH-induced neural plasticity has been reported for both respiratory and nonrespiratory-related motor outputs. The goal of the present study is to characterize possible similar effects of AIH on the external oblique (EO), an expiratory abdominal muscle active during micturition. To this end, bladder intravesical pressure along with external urethral sphincter (EUS) and EO EMG activity were recorded in urethane-anesthetized (1.4 g/kg), spontaneously breathing adult female Sprague Dawley rats. In addition, EMG recordings were acquired from the diaphragm to identify periods of inspiration and expiration and to assess AIH-induced respiratory LTF. Reflex micturition events were elicited by continuous infusion of saline into the bladder, and the rate of infusion was adjusted to achieve a baseline bladder inter-contraction interval of approximately 4 minutes. Following 30-40 minutes of breathing room air (21% O2; baseline recording), rats were exposed to a single bout of AIH consisting of three five-minute episodes of hypoxia (10% O_2; 90% N_2) each separated by five-minute exposures to room air. Data acquisition continued for at least 90 minutes following the AIH exposure. EO activity was associated with both micturition and respiration and occurred primarily at the peak of bladder contraction during the period of EUS bursting (i.e., active voiding). Further, during EO activation tonic activity as well as phasic discharges during expiration were observed. During baseline recording, the amplitude and duration of EO activity varied both within and between rats. During AIH, EO activity was attenuated, particularly during the episodes of hypoxia. However, EO activity was markedly facilitated (both amplitude and duration) for up to 60 minutes following AIH. Sustained EO LTF was also observed in rats that exhibited little or no EO activation during baseline recording. In every case, the enhanced EO activation following AIH continued to be associated with active micturition and exhibit both tonic and/or phasic expiratory patterns of activity. These results are consistent with the

hypothesis that AIH initiates a generalized systemic motor LTF that includes spinal somatic motor systems.

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Poster

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SEP-SES-DGESU2016

Title: Histomorphometry of postganglionic and sensory neurons of the vagina of nulliparous and pregnant rats

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Abstract: The female reproductive organs undergone anatomical and histological changes associated with their reproductive status. The aim of this study was to describe the morphological features of urogenital tract in pregnant and virgin rats. Five pregnant and four virgin rats were studied. The vaginal wall of 17-18 day of pregnancy or virgin rat was injected with 5 µl of horseradish peroxidase wheat germ agglutinin (HRP) and after 48/72 hours of injection, the left major pelvic ganglia (MPG) and the right dorsal root ganglia (DRG's) were obtained. We founded that the postganglionic neurons innervating the vagina are localized in the middle region of the MPG. The vaginal sensory neurons were mainly founded in the L6 and S1 DRG's. The pregnancy did not modify the number of vaginal postganglionic neurons (862.0±42.4 *vs* 849.0±52.30, p=0.8) neither their morphometric characteristics: soma area (pregnant 90.7±2.1 μm^2 *vs* virgin 134.9±2.3μm^2, p=0.1), diameter (11.9±0.3 µm *vs* 13.6±1.1µm, p=0.4). The pregnancy did not modify the number of the sensory neurons (L6, 35.5±15.8 *vs*

virgin 46.8±30.7, t=21 p=0.9; S1, 64.6±37.4 *vs* 75.1±68.5, t=19, p=0.9), neither their morphometric features: soma area (L6 = 440.1±76.1 μm^2 *vs* 391 ±43.4 μm^2, p=0.3; S1 (371.0 ±36.9 μm^2 *vs* 377.2 ±12.5 μm^2, p=0.9); diameter (L6, 26.3± 1.4 µm *vs* 24.7±0.4 µm, p=0.2 and S1, 24.03±1.9 µm *vs* 25.1±0.9 µm, p=0.7). Our results showed that despite the several physiological and morphological pregnancy related changes, and in contrast to the described reduction in the vaginal innervation in pregnant rats, there is no evidence of anatomical changes in autonomic or sensory vaginal neurons.

Disclosures: **N. Mirto-Aguilar:** None. **N. Xelhuantzi:** None. **J. Palacios:** None. **M. Juarez:** None. **Y. Cruz:** None.

Poster

448. Gastrointestinal: Reproductive Regulation

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Topic: F.07. Autonomic Regulation

Support: NIH NIDDK DK056132 12-16

Title: Modulation of blood glucose by the dorsal vagal complex

Authors: ***C. R. BOYCHUK**, J. A. BOYCHUK, K. C. HALMOS-SMITH, B. N. SMITH; Physiol., Univ. of Kentucky, Lexington, KY

Abstract: The brainstem dorsal motor nucleus of the vagus (DMV) contains the preganglionic parasympathetic motor neurons that provide motor output to most subdiaphragmatic viscera important in regulating metabolism. The DMV serves as the final central modulatory point for descending parasympathetic activity, and its activity is tightly controlled by GABAergic inhibitory synaptic input arising from the nucleus tractus solitarius (NTS). Thus inhibitory, GABAergic neurotransmission from the NTS contributes significantly to parasympathetic visceral control. Together with area postrema, the DMV and NTS make up the dorsal vagal complex (DVC), a brainstem site critical in mediating the gut-brain-liver circuit controlling systemic [glucose]. Specifically, GABA neurons in the NTS are glucose sensing, and elevated glucose increases GABA neurotransmission in the DMV. Chronic hyperglycemia also induces a variety of neuroplasticity events within the DVC. Therefore, we hypothesized that modulating GABA neuron activity in the DVC causes changes in peripheral blood [glucose] through an efferent vagal pathway. This hypothesis was tested by integrating in vitro electrophysiology with acute drug infusion and chemogenetics using designer receptors exclusively activated by designer drugs (DREADDs) in the whole animal. Preliminary electrophysiological results

confirm previous reports that GABA neurons in the NTS respond to changes in [glucose] through specific intracellular mechanisms, including glucokinase (GCK) activation. Acute local infusion of a $GABA_A$ receptor agonist or a GCK activator indicated that modulating glucose sensitivity or GABA receptors in the DVC can elevate blood [glucose]. Chemogenetic manipulation of GABA neurons in the DVC also resulted in significant changes in blood [glucose]. After chronic hyperglycemia (3-5 days), both electrophysiological and in vivo data suggest that these GABAergic vagal circuits undergo significant plasticity. These studies are the first to demonstrate that GABAergic signaling in the DVC regulates systemic glucose homeostasis. Defining the glucoregulatory functions of the DVC provides a fresh perspective on our understanding of autonomic control of energy homeostasis and will likely translate to novel therapeutic targets for diabetes.

Disclosures: **C.R. Boychuk:** None. **J.A. Boychuk:** None. **K.C. Halmos-Smith:** None. **B.N. Smith:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: NIH grant R01DK106181

Title: Spinal cord stimulation may improve the voiding function in rodent with dopaminergic brain lesion injury

Authors: J.-C. YEH, J. MAO, *H. H. CHANG;
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Abstract: Neurodegenerative disorders often have an impact on urodynamics; Parkinson's disease (PD) is one of said disorders that specifically involves voiding dysfunction. Spinal cord stimulation (SCS) is currently used as a therapeutic option in the clinic for the management of neuropathic pain and spasticity. As it relates to urodynamic studies, the SCS technique has been successful for promoting micturition through reducing urethral resistance in the rodent model of spinal cord injury. The present study aimed to examine the effect of SCS on micturition following a dopaminergic brain lesion in rodents that mimics PD. Female Sprague-Dawley rats underwent injection of 6-OHDA (n=4) or vehicle (n=4) at four injection sites bilaterally targeting the dorsal striatum. SCS was then performed directly between the L2 and L6 spinal levels at 28 days following the injury. Cystometry and external urethral sphincter (EUS) electromyography

were obtained while the rats were under urethane anesthesia. Prior to the SCS the PD rats showed detrusor over-activity and a significant increase in the maximum intravesical pressure (IVPmax); the amplitude and area under the curve (AUC) of EUS activity during filling and voiding were increased as compared with the sham rats. Following the baseline recordings, the bladder was then infused with room temperature saline to distend it to a volume of over 60% of bladder capacity. SCS was then performed at the L2-6 spinal levels for the duration of 1 min (40 Hz, 0.2 ms pulse duration, and 2-6 volts). The evoked voiding contraction was observed approximately 30-65 seconds after SCS. The IVPmax, amplitude, and the AUC of EUS activity during voiding were all significantly decreased in the PD rats as compared to the spontaneous voiding contractions as recorded without the aid SCS; IVPmax, amplitude, and the AUC of EUS for PD rats following SCS more closely resembled the data collected for the sham rats. The preliminary results indicate that SCS on the spinal levels of L2-6 in the rats with dopaminergic brain lesion could elicit the bladder contractions accompanying decreased EUS activity. Implications are that SCS on the spinal levels of L2-6 could potentially improve voiding function through the reduction of urethral resistance in patients with Parkinson's disease.The authors thank Dr. DP Holschneider's Laboratory at USC for assisting with the surgical injection of 6-OHDA.

Disclosures: **J. Yeh:** A. Employment/Salary (full or part-time): Institute of Urology, University of Southern California. **J. Mao:** A. Employment/Salary (full or part-time): Institute of Urology, University of Southern California. **H.H. Chang:** A. Employment/Salary (full or part-time): Institute of Urology, University of Southern California. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIH grant (R01DK106181).

Poster

448. Gastrointestinal: Reproductive Regulation

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Topic: F.07. Autonomic Regulation

Support: NIH Grant DK056132

Title: Uncontrolled diabetic hyperglycemia is resolved by vertical sleeve gastrectomy in a murine model of type 1 diabetes

Authors: *K. HALMOS, C. R. BOYCHUK, B. N. SMITH;
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Abstract: Recent evidence supports the emerging hypothesis that brainstem autonomic neurons are affected by and contribute to systemic glucose regulation. Provocatively, activation of vagal afferent terminals at the level of the gut results in altered blood [glucose] in a manner that involves a brain-centered glucose regulatory system. Moreover, most GABAergic neurons in the brainstem nucleus tractus solitarius (NTS), which receive primary vagal afferent synaptic information, are sensitive to changes in [glucose] in a glucokinase (GCK)-dependent fashion and GCK expression and glucose-sensitivity are reduced in the NTS after several days of continuous hyperglycemia in the streptozotocin (STZ)-treated mouse. Vertical sleeve gastrectomy (VSG) and other bariatric surgeries can result in resolution of type 2 diabetes in humans and animal models, but information about effects of VSG on type 1 diabetes is scarce. Here, we used the STZ-treated mouse model of type 1 diabetes to determine effects of VSG on uncontrolled hyperglycemia, brainstem GCK expression, and markers of hepatic gluconeogenesis to test the general hypothesis that an insulin-independent, brain-centered glucose regulatory system contributes to systemic glucose regulation after bariatric surgery. Uncontrolled hyperglycemia (>300 mg/dl) was maintained for 3-5 days in STZ-treated mice, at which time VSG or sham surgery was performed. After VSG, blood [glucose] was normalized in ~80% of mice, an effect that was evident within three days and persisted for >4 weeks. Correspondingly, molecular expression of GCK in the vagal complex, which is reduced after several days of hyperglycemia, was reinstated to control levels after VSG. These outcomes were independent of reduced caloric intake after VSG and were mimicked by insulin treatment in STZ-treated mice. Indicators of hepatic gluconeogenesis (e.g., PEPCK, G6P) were significantly altered after STZ treatment and were reinstated to normal levels after VSG. Thus, VSG resolves uncontrolled hyperglycemia in a manner that involves reinstatement of glucose-sensitivity in the vagal complex in this model of type 1 diabetes. These data are consistent with a role for brainstem control of systemic glucoregulation involving a vagally-mediated gut-brain-liver circuit.

Disclosures: **K. Halmos:** None. **C.R. Boychuk:** None. **B.N. Smith:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: CONACyT YCG:183446

CONACyT RJM:328833

Title: Sensory urethral innervation in male rats

Authors: *R. JUAREZ MENDIETA[1], I. JIMENEZ[2], Y. CRUZ[1];
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[2]Ctr. de Investigación y Estudios Avanzados, Inst. Politécnico Nacional, México, Mexico

Abstract: In rats, the male urethra is divided into four regions: prostatic, membranous, diverticulum and penile urethral. Approximately half of the urethra is surrounded by the external urethral sphincter (EUS). The aim of the present study was to determine the afferent innervation of the urethra of the male rat. In the Experiment one, the reflex EUS electromyographic (EMG) activity induced by urethral stimulation was used as indicator of afferent innervation. The EUS EMG activity was recorded in eight animals during mechanical stimulation of the penile urethra and prostatic urethral regions, before and after the neurectomy of the sensory branch of the pudendal nerve (SBPdN) or the viscerocutaneous branch of the pelvic nerve (VBPvN). In the Experiment two, evoked action potentials were recorded in the dorsal roots of L5, L6 and S1 segments during mechanical or electrical stimulation of the urethra. The Experiment one results showed that the SBPdN neurectomy eliminated the reflex activity of the EUS induced in response to stimulation of the penile urethra. The EUS EMG activity induced during stimulation of the prostatic urethra decreased ($p<0.05$) in amplitude and frequency after the SBPdN neurectomy. In contrast, the neurectomy of the VBPvN increased the EUS EMG activity induced in response to stimulation of the penile urethra, but eliminated the EMG response to prostatic urethral stimulation. In the Experiment two was found that mechanical or electrical stimulation of the penile or prostatic urethra evoked action potentials in the L6 dorsal roots. When the penile urethra was stimulated at 20 and 15 mm deep, the first component of the potential appeared with a latency of 1.6 ms ± 0.08 and 1.5 ± 0.08 ms, respectively. We conclude that the urethral afferents of the male rat travel through the VBPvN and the SBPdN, with the latter as the main pathway. The urethral sensory information is mostly integrated at the L6 spinal cord segment.

Disclosures: R. Juarez Mendieta: None. **I. Jimenez:** None. **Y. Cruz:** None.

Poster

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NYS Spinal Cord Injury Research Board C30601 (JSC)

Title: Development of skull-mounted port for bladder infusion during cystometry in unanesthetized, freely-moving rats

Authors: *J. S. CARP, J. R. WOLPAW;
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Abstract: Cystometry for evaluation of lower urinary tract (LUT) function is often performed in awake, restrained animals with implanted catheters to avoid the influence of anesthesia. Because the stress of restraint may influence LUT function, we are developing methods for infusing saline into the bladders of awake, freely-moving rats and comparing their cystometric data with those of head-restrained rats. Female rats (n=4) were each implanted with a suprapubic catheter secured in the bladder dome. The catheter was routed subcutaneously to a Delrin access port (22 mm high x 10 mm diameter) with a silicone rubber septum mounted on the skull with dental cement and screws. In two rats, a bolt was attached to the skull to allow the animals to be restrained by securing the bolt to a rigid frame. Drinking water contained 5-7 mM trisodium citrate to reduce precipitate formation around the implanted catheter tip and in the bladder. After a 1-2 week recovery, rats were placed periodically in large cages and a 19-ga Huber needle was inserted through the port septum to allow continuous infusion of room-temperature sterile saline at 0.2 ml/min into the bladder while measuring pressure. Recording sessions lasted 30-60 minutes, after which each rat received a 0.5-ml injection of a citrated antibiotic lock solution into the port. To date, recordings have been made from two freely moving rats (9 and 5 sessions over 8 and 4 weeks, respectively) and two head-restrained rats (5 and 3 sessions over 7 and 2 weeks, respectively). Although pressure artifacts were evident during movement, these disappeared when the rats assumed a quiet quadrupedal stance during voiding, during which contraction pressure (CP), inter-contraction interval (ICI), and contraction duration (CD) were determined. Comparing freely-moving and head-restrained rats, ICI was similar (5 vs 6 min, respectively), CP was higher (35 vs 28 mm Hg, respectively), and CD was shorter (9 vs 15 s, respectively). These results suggest the feasibility of maintaining a patent fluid catheter to the bladder for several weeks, allowing longitudinal study of LUT function in the same subject. Further study will determine how long the recordings remain viable, and whether addition of citrate to drinking water and the port promote catheter patency.

Disclosures: J.S. Carp: None. **J.R. Wolpaw:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: USDA Hatch ILLU-538-926

University of Illinois FIRE grant

Title: Effect of parasympathetic or sympathetic denervation on intestinal epithelial stem cell proliferation

Authors: *E. A. DAVIS[1], M. C. WASHINGTON[3], H. PHILLIPS[2], A. I. SAYEGH[3], M. J. DAILEY[1];
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Abstract: The mammalian intestinal epithelium is critical for nutrient absorption, hormone release and immune function. Stem cells located in the intestinal epithelial crypts proliferate and differentiate to produce mature cells that serve these functions. Via this process, complete turnover of the epithelium occurs every few days. Previous studies show that the parasympathetic (PNS) and sympathetic (SNS) nerves play a role in this turnover process, as surgical ablation of each branch alters stem cell proliferation. These studies, though, did not control for the surgically-induced decreases in food intake and did not investigate segmental differences in autonomic control along the proximal to distal axis of the intestine. Given that we have found that the amount of food intake can dictate the rate of stem cell proliferation and that there are anatomical differences in PNS and SNS innervation along the intestinal axis, we wanted to evaluate the PNS and SNS control of intestinal stem cell proliferation independent of changes in food intake and in each segment of the intestine. Adult male Sprague Dawley rats underwent 1) bilateral subdiaphragmatic vagotomy to denervate PNS nerves (n=12) or 2) bilateral subdiaphragmatic celiacomesenteric ganglionectomy to denervate SNS nerves (n=12). Control groups for both surgical denervations included 3) a sham surgery, *ad libitum* fed group (n=8) and 4) a sham surgery, pair-fed group that was fed equal kcals to the surgical group to control for any surgical-induced changes in food intake (n=8). Three weeks after surgery, animals were given an intraperitoneal bromodeoxyuridine (BrdU) injection and sacrificed 6 hours later. Intestinal tissue was excised and immunohistochemically processed to visualize BrdU positive cells, an indication of cell proliferation. PNS denervation induced an expected decrease in food intake, but Brdu positive cells and
the proliferation ratio (positive cells/total crypt cells) were significantly increased compared to the sham, pair-fed animals ($p<0.05$). Segmental differences show a pronounced effect in the jejunum, but no significant difference in the ileum. SNS denervation resulted in a significant increase in the proliferation ratio compared with the sham surgical group in the duodenum and jejunum, but not in the ileum ($p<0.05$). These data suggest that anatomical differences in PNS and SNS intestinal innervation result in a differential effect on stem cell proliferation, leading to changes in the regenerative capacity of the epithelium and integrity in the tissue function.

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Poster

448. Gastrointestinal: Reproductive Regulation

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Topic: F.07. Autonomic Regulation

Support: NIH grant DK102367

Title: Network dynamics underlying the encoding of visceral sensorimotor information

Authors: *A. MANOHAR[1], A. CURTIS[2], S. ZDERIC[2], R. VALENTINO[2];
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Abstract: Neural networks with bi-directional communication between the brain and viscera are critical for regulating bladder function. Urodynamic status must be communicated to distributed brain regions that increase arousal, redirect attention and modify behavior prior to micturition. Despite its importance, the process by which the brain encodes visceral signals is relatively unexplored. Barrington's nucleus (BN), in the pons, initiates micturition through projections to preganglionic parasympathetic neurons that innervate the detrusor. BN neurons can coordinate peripheral and central components of micturition through its projections to the norepinephrine nucleus, locus coeruelus (LC), which innervates the prefrontal cortex (PFC) that governs executive function. Using multisite recordings we sought to understand the central encoding of visceral sensory information that yields the appropriate behavioral output. Rats were implanted with multi-wire electrodes to record single unit activity and/or local field potentials in the PFC and either the BN or LC. Neuronal and network activity were recorded simultaneously with bladder pressure (cystometry) in awake freely behaving animals. LC neuronal discharge showed a multi-fold increase approximately 30s prior to micturition threshold and this was temporally linked to PFC desynchronization. At this time LC network activity shifted into a prominent theta oscillation that was associated with PFC desynchronization and LC-PFC coherence was increased specifically in the theta band. The initiation of LC theta oscillations may be a signal to shift attention and initiate voiding behaviors prior to micturition. Further, contrary to the notion that BN functions as a switch, BN neurons discharged spontaneously and exhibited synchronized bursting at different frequencies occurring right before and after the peak in the bladder pressure suggesting an underlying code for sensory and motor aspects of visceral function. Together, these results underscore the complex synchronization of pontine and cortical neurons with bladder afferent information that is required to maintained coordination between voiding behavior and urination. Dysregulation of this network could be a prominent signature underlying bladder pathology.
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Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Title: Reflexive inhibition of bladder function via saphenous nerve stimulation in anesthetized rats

Authors: *Z. MOAZZAM, P. B. YOO;
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Abstract: Overactive bladder (OAB) is characterized by symptoms of urinary frequency, urgency and incontinence. Percutaneous tibial nerve stimulation (PTNS) is a minimally invasive yet effective treatment where electrical pulses are delivered at 20 Hz coupled with amplitudes set below the foot twitch threshold (< 1T). However, recent animal studies report that tibial nerve mediated bladder reflexes require stimulation amplitudes exceeding 2T. The discrepancies in clinical and physiological data prompted the current study where we hypothesized the presence of a low-threshold bladder-inhibitory pathway that is co-activated during clinical PTNS therapy. Given the anatomical placement of the percutaneous electrode, we conjecture contributions from the saphenous nerve (SAFN) as a potential mechanism for PTNS-mediated therapeutic efficiency. We conducted 77 stimulation trials in 19 urethane-anesthetized adult rats (Sprague-Dawley) with urodynamic fills. The SAFN trunk was instrumented caudal to the knee joint where a bipolar stimulating nerve cuff electrode was implanted. Bladder function was characterized by changes in multiple urodynamic parameters (e.g., bladder contraction rate (BCR)). Finite durations of electrical pulses were applied at low amplitudes (i.e., 25 μA) while the frequencies ranged between 2 Hz - 50 Hz. The pulse width was set at 200 μs for all trials. Our findings suggest that SAFN stimulation at low amplitudes can effectively inhibit on-going bladder activity at all frequencies tested. Short (10 min) stimulation trials conducted at 20 Hz resulted in the most consistent changes during both intra-stimulation (50.2±4.7% decrease in BCR) and post-stimulation (38.7±5.9% decrease in BCR). The inhibitory effects of 10 minute stimulation trials were observed to abate shortly following termination of the stimulus. Conversely, prolonged SAFN stimulation (40 min) at 10 Hz significantly decreased the average

evoked contraction amplitude (49.0±10.5%) along with an increase in the voiding threshold (17.6±4.8%) during stimulation leading to complete inhibition of periodic bladder contractions during the post-stimulation phase. Such strong inhibitory effects were observed to last for an approximate period of 40 - 50 minutes post-stimulation. These novel pre-clinical findings suggest the presence of a highly sensitive reflex pathway that can elicit strong and consistent bladder inhibitory effects during both acute and prolonged conditions. Future work is needed to fully characterize this reflex pathway and determine the clinical feasibility of translating SAFN stimulation as an efficacious neuromodulation alternative for OAB patients.

Disclosures: Z. Moazzam: None. **P.B. Yoo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent file has been submitted.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Support: CONACyT YCG 183446

CONACyT JAH 309019

SEP-SES-DGESU2016

Title: Unilateral denervation of the genitourinary tract induces signs of sexual, urinary dysfunction and infertility in male rats

Authors: J. ARELLANO[1], F. CASTELÁN[2], J. CUATECONTZI[2], *Y. CRUZ[2];
[1]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico; [2]Univ. Autonoma Tlaxcala, Tlaxcala, Mexico

Abstract: Introduction and Objectives. The urogenital tract (UGT) is the anatomical substrate of micturition, sexual behavior and fertility. In male rats the autonomic innervation of the UGT is mainly provided by the major pelvic ganglion (MPG), which innervates the bladder, urethra and sexual accessory glands. The somatic component is represented by the motor branch of the sacral plexus (MBSP), which innervates the external urethral sphincter and the perineal muscles (ischiocavernosus and bulbospongiosus). The objective of this study was to determine whether autonomic or somatic unilateral denervation of the UGT induces signs of urinary/sexual dysfunction and/or infertility.

Methods. Eighteen adult Wistar male rats were maintained on a 12/12 light/dark cycle with food and water provided *ad libitum*. The animals were sexually trained and assigned to the following groups (six animals per group): left MPG ablation (MPGA), left MBSP transection (MBSPT) or sham surgery (SH). Urinary and sexual functions were evaluated before, as well as at 1 and 3 weeks after surgery. Fertility was determined at 12 weeks post-surgery. A closed circuit video with infrared cameras was used to record micturition and copulatory parameters. Urinary function was tested during the last 6 h of the dark phase by placing rats in a wired-floor cage and voided urine was collected in a plastic container. Sexual behavior was assessed by placing the male rat with a receptive female rat in a Plexiglas cylinder arena. Fertility was evaluated by determining the percent of pregnant females housed with a male for two weeks.
Results. Pre-surgery the rats presented ~ 5 voids/6h. Three weeks after the surgery voiding frequency in the MPGA and MBSPT animals increased (SH=7$\pm$1.3; MPGA=10.55$\pm$0.9; MBSPT=12.5$\pm$1.7; $p<0.05$), while voiding volume decreased significantly (SH=0.66$\pm$0.17; MPGA=0.4$\pm$0.05; MBSPT=0.3$\pm$0.04; $p<0.05$). In the MBSPT group, 20% of animals did not reach ejaculation. The number of intromissions decreased in both denervated groups ($p<0.05$) and fertility was only affected in MBSPT (63% of pregnant females). Conclusion. Unilateral autonomic or somatic denervation of the UGT induces signs of urinary and sexual comorbidity in male rats.

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Poster

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Topic: F.07. Autonomic Regulation

Support: University of Michigan MCubed Pilot Grant

Title: Modulation of urine glucose by renal nerves stimulation in rat

Authors: ***A. A. JIMAN**[1,2], A. G. LEWIS[3], K. H. CHHABRA[4], P. S. CEDERNA[1,3], R. J. SEELEY[3], M. J. LOW[4], T. M. BRUNS[1,2];
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Abstract: Diabetes is a growing global disease. One of the main natural body adaptations to high blood glucose levels caused by diabetes is glycosuria. Glycosuria is a condition where excess blood glucose is excreted into urine. In recent years, the role of kidney (renal) nerves in blood

glucose control through glycosuria has been identified. We hypothesized that renal nerve activity can be influenced by electrical stimulation to regulate glycosuria. In this study, renal nerves of the left kidney in male Long-Evans rats were accessed and encircled by a nerve cuff electrode. Ureters of the left and right kidneys were cannulated to obtain separate urine samples. Electrical stimulation (2 Hz, 10 V) was applied on renal nerves of the left kidney for 30-40 minutes. A bolus dose of glucose was administered through the jugular vein after 2 minutes into stimulation. Urine samples were collected at 5- or 10-minute intervals and an assay kit was used to quantify urinary glucose excretion. Across sampling periods the stimulated left kidney generally had much less total urine glucose excreted, typically 45-94% lower than the unstimulated right kidney output. Our results so far suggest that renal nerves stimulation can modulate urinary glucose excretion. Future experiments are required to confirm the effects of renal nerves stimulation on glycosuria and further explore the stimulation parameter space.

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Poster

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Topic: F.07. Autonomic Regulation

Support: GlaxoSmithKline

Title: Prostaglandin E2 installation as an overactive bladder model in cats

Authors: ***C. L. LANGDALE**[1], J. A. HOKANSON[1], A. SRIDHAR[5], W. M. GRILL[1,2,3,4]; [1]Biomed. Engin., [2]Electrical and Computer Engin., [3]Neurobio., [4]Surgery, Duke Univ., Durham, NC; [5]Bioelectronics R&D, GlaxoSmithKline, Stevenage,, United Kingdom

Abstract: Overactive bladder (OAB), as described by the International Continence Society, is defined as urgency, with or without urge incontinence, usually with frequency and nocturia. Animal models are essential for advancing our understanding of bladder function as well as developing novel therapeutic treatments for OAB. Due to the inability to identify urgency in animals, symptoms such as detrusor over-activity, frequency, and voiding parameters (bladder capacity (BC) etc…) are used as proxies for the symptoms of OAB. There are a number of animal models in rats and mice that result in symptoms of OAB (induced hypersensitive/irritation, genetic, bladder outlet obstruction, and neurogenic models). However, OAB models in the cat, which is commonly used to evaluate lower urinary tract function due to

the similarity to humans, are limited. Acetic acid (AA)-induced bladder irritation model in the α-chloralose anesthetized cat has been routinely used as an OAB model, and produces symptoms of OAB including increased frequency of voiding and decreased BC. However, an apparent limitation to the AA infusion model is the associated damage to the bladder urothelium and urethral epithelium, which may have unintended consequences. Alternative OAB models for evaluating therapeutic targets in cats are needed. We hypothesized that intravesical infusion of PGE2, a rat OAB model, will cause OAB symptoms similar to acetic acid infusion in cats. Acute α-chloralose (65 mg/kg) anesthetized female cats were studied using in vivo cystometry (CMG). A PE-90 catheter was placed to measure bladder pressure and for intravesical infusion of saline and PGE2 (1, 5, and 10 μM). Bipolar electrodes were placed on the EUS to record electromyogram (EMG) activity during CMGs. In a subset of experiments, pelvic nerve (PEL) stimulation was delivered after 5 μM PGE2 infusion at different amplitudes (0.8, 1.0, and 2.0 times evoked PEL-EUS reflex threshold) and frequencies (1 and 10 Hz). PGE2 dose dependently decreased BC, with a 50% reduction in BC at 5 μM. No apparent changes in either EUS EMG or voiding efficiency were noted. Additionally, PEL stimulation restored BC in an amplitude dependent manner. These findings suggest that PGE2 may be an alternative OAB model to evaluate therapeutic targets in cats.

Disclosures: **C.L. Langdale:** 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; GlaxoSmithKline. **J.A. Hokanson:** 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; GlaxoSmithKline. **A. Sridhar:** A. Employment/Salary (full or part-time): GlaxoSmithKline. **W.M. Grill:** 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; GlaxoSmithKline.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Program#/Poster#: 449.05/GGG2

Topic: F.07. Autonomic Regulation

Support: NIH Grant U18EB021793

Title: Wireless monitoring and optogenetic modulation of bladder function

Authors: ***A. D. MICKLE**[1], J. YOON[2], S. M. WONG[2], S. PARK[2], K. N. NOH[2], K. MEACHAM[1], J. ROGERS[2], R. W. GEREAU, IV[1];
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Abstract: Millions of people in the United States suffer from bladder dysfunction and pain caused by interstitial cystitis/bladder pain syndrome and overactive bladder. The underling pathologies for many of these diseases are poorly understood and this is the primary reason most current treatments are ineffective. To address this issue, we have designed and tested an implantable wireless optoelectronic system to monitor and modulate bladder function. This new technology eliminates the need for implantation of potentially-damaging bladder catheters or electrodes, and provides unique access to bladder functionality in the awake, freely-moving rat. We utilized a combination of viral delivery of opsins and a novel strain gauge that measures dynamic changes in bladder circumference, to modulate and monitor bladder function, respectively. Our hyper-conformal strain gauge wraps around the bladder and as the bladder expands changes in geometry of the device linearly increases resistance. We show that this change in resistance correlate to traditional bladder activity measurements like intravesicular pressure. We attached microscale light emitting diodes (LED) to the strain gauge that can be used to activate light-sensitive opsins for optogenetic regulation of neuronal activity. The strain gauge and LEDs connect with an implantable Bluetooth base station that allows for wireless control and monitoring of bladder activity. Implementation of this new technology will provide unique access to understanding bladder functionality without need for implantation of potentially damaging bladder catheters or electrodes. This technology could thus lead to novel insights into the mechanisms of bladder control and pain. Additionally the refinement of this novel technology and virally delivery methods for optogenetic channels could lead to development of future therapies for bladder dysfunction. This work was funded by a grant from the NIH Common Fund's SPARC program, U18EB021793 to RG and JR.

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Poster

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Program#/Poster#: 449.06/GGG3

Topic: F.07. Autonomic Regulation

Support: NIH R01 Grant DK095817

Title: Autonomic control of bladder function is regulated by TREK-1, a two-pore domain potassium channel

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Abstract: *Background.* Regulation of urinary bladder function depends on coordinated activity between the nerves and smooth muscle cells in the bladder wall. The mechanisms of mechanosensitivity and mechanotransduction during the storage and voiding phases of the micturition cycle are currently not well understood. Previously, we characterized the expression and function of two-pore domain potassium (K_{2P}) channels in the normal human bladder, and confirmed that TREK-1 is the predominantly expressed member of the family on both bladder smooth muscle cells and bladder sensory neurons. In the present study, we sought to determine the key regulatory elements of the cell membrane and cytoskeleton which affect TREK-1 expression and function in the human urinary bladder, as well identify any differences in TREK-1 between normal and overactive urinary bladders. *Methods:* We used freshly dissociated detrusor smooth muscle cells obtained from 17 human donors and patients with detrusor overactivity (DO). Molecular (RT-PCR and Western), histological, immunohistochemical, electrophysiological (whole-cell patch-clamp) and pharmacological approaches were used to evaluate regulatory mechanisms affecting expression and/or function of TREK-1 channels in the human bladder. *Results:* Lower levels of TREK-1 expression were detected in patients with DO in comparison with normal bladders. The average level of expression in control bladders was twice higher than in the DO group ($p \leq 0.05$). Expression of TREK-1 was also tested in single isolated bladder smooth muscle cells picked up by patch pipette with large diameter to avoid contamination by other cells types (neurons, macrophages etc). The changes in channel expression correlated with a distortion in caveolin patterns suggesting a possible connection between the channel and cell cytoskeleton. *Conclusion:* Decreased expression of TREK-1 channel in the human detrusor is associated with detrusor overactivity. Physiologically, these changes are associated with smaller bladder volumes, decreased bladder capacity and development of the sensation of urgency, all of which are often observed in patients with overactive bladder. This research is supported by NIH R01 grant DK095817 (to APM).

Disclosures: R.H. Pineda: None. **R.B. Meacham:** None. **A.P. Malykhina:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: NIDDK K12 DK100024

GSK Bioelectronics Research Program

Title: OAB ithout an overactive bladder: insights from an acute prostaglandin E2 rat model

Authors: *J. A. HOKANSON[1], C. LANGDALE[1], A. SRIDHAR[2], W. GRILL[1];
[1]Biomed. Engin., Duke Univ. Dept. of Biomed. Engin., Durham, NC; [2]GlaxoSmithKline, Stevenage, United Kingdom

Abstract: Animal models of overactive bladder (OAB) enable the testing of therapies to treat the symptoms of OAB. Intravesical administration of Prostaglandin E2 (PGE2) was previously proposed as a model of OAB. Administration of PGE2 intravesically in healthy women causes a strong desire to void. In rat models intravesical PGE2 administration decreases bladder capacity, but its mechanism of action has not been well explored.

Female Wistar rats aged 15 - 23 weeks were anesthetized (urethane, 1.2 g/kg S.C.) and single fill cystometrograms were conducted with saline or PGE2 (100 μM). Wires were inserted percutaneously into the pelvic floor to record the activity of the external urethral sphincter (EUS). Additional experiments were conducted with suture placed around the bladder neck to allow administration of PGE2 exclusively to either the bladder (isovolumetric preparation) or the urethra.

Intravesical administration of PGE2 decreased bladder capacity (-17 ± 7.5%, p = 0.040, n=10). This decrease coincided with, and was sometimes preceded by, an increase in EUS EMG during bladder filling.

There was no clear increase in the number of large non-voiding contractions or change in bladder compliance (p=0.62, n = 10) following PGE2 installation. In isovolumetric preparations in which PGE2 was administered only to the bladder there was no change in the bladder capacity (p = 0.10, n = 7).

Infusing PGE2 into the urethra led to a large decrease (69% ± 10%, n = 3) in urethral infusion pressure, signaling a dramatic decrease in urethral tone.

These results suggest that urethral relaxation, not bladder activation, is driving the decrease in bladder capacity following intravesical PGE2 administration. The increase in EUS EMG may be compensatory to try to maintain continence during bladder filling.

Targeting the urethra, particularly urethral smooth muscle, may be a promising new avenue for the design of drugs and devices that treat OAB.

Disclosures: **J.A. Hokanson:** None. **C. Langdale:** None. **A. Sridhar:** None. **W. Grill:** None.

Poster

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Title: GABA, glycine and opioid neurotransmitter mechanisms underlying sacral neuromodulation of bladder overactivity in cats

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Abstract: The aim of this study was to examine GABA, glycine and opioid neurotransmitter mechanisms underlying the inhibition induced by sacral neuromodulation (SNM) of nociceptive bladder overactivity in cats under α-chloralose anesthesia. Initial bladder capacity was determined by repeated cystometrograms (CMGs) using saline infusion. Intravesical infusion of 0.5% acetic acid (AA) irritated bladder and induced nociceptive bladder overactivity. AA irritation significantly ($P<0.01$) reduced bladder capacity to 59.5±4.8% of saline control. S1 or S2 dorsal root stimulation at threshold intensity for inducing leg or anal twitch inhibited bladder overactivity and significantly ($P<0.01$) increased bladder capacity to 105.3±9.0% and 134.8±8.9% of control, respectively. Picrotoxin (a $GABA_A$ receptor antagonist, i.v.) blocked S1 inhibition at 0.3 mg/kg and removed S2 inhibition at 1.0 mg/kg. Picrotoxin (0.4 mg, i.t.) had no effect on sacral inhibition, but produced significant ($P<0.05$) bladder inhibition after the stimulation. Naloxone (an opioid receptor antagonist, 0.3 mg, i.t.) significantly ($P<0.05$) reduced bladder capacity and removed the post-stimulation inhibition unmasked by i.t. picrotoxin. Strychnine (a glycine receptor antagonist, i.v.) at 0.03-0.3 mg/kg significantly ($P<0.05$) increased AA control bladder capacity but did not change sacral inhibition. Picrotoxin (0.3 mg, i.v.) after strychnine (0.3 mg, i.v.) significantly ($P<0.05$) increased AA control bladder capacity and completely removed sacral inhibition. These results indicate that $GABA_A$ receptors play an

important role in sacral neuromodulation of nociceptive bladder overactivity at a supraspinal site, while glycine receptors are not involved. Opioid receptors play a role in post-stimulation inhibition unmasked by blocking spinal $GABA_A$ receptors.

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Poster

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Topic: F.07. Autonomic Regulation

Support: Neilsen Foundation Grant 314980

Title: Chronic monitoring and stimulation of the lower urinary tract during sedated and awake testing

Authors: ***S. E. ROSS**, A. OUYANG, A. KHURRAM, A. A. A. JIMAN, Z. J. SPERRY, C. J. STEPHEN, T. M. BRUNS;
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Abstract: Patients with bladder dysfunction refractory to standard treatments may benefit from a closed-loop neuroprosthesis for bladder control. A greater understanding of lower urinary tract (LUT) neurophysiology can inform the development of such a device. While a plethora of information exists on acute LUT nerve interfaces, literature on long-term interfacing with LUT neurons is lacking. To this end, we are developing a chronic interface that integrates monitoring of bladder pressure and LUT activity with stimulation of peripheral pathways. Under isoflurane anesthesia, penetrating microelectrodes (32-channel arrays, Blackrock Microsystems) are implanted in sacral dorsal root ganglia (DRG) at the S1 and S2 levels and cuff electrodes are placed on the pudendal nerve. Supra-pubic bladder catheters for saline infusion and bladder monitoring are also implanted. Electrode and catheter connectors are tunneled subcutaneously to the midline rostral to the tail and enclosed in a custom-made external housing mounted percutaneously to the iliac crests. After recovery from surgery, animals are tested for ~2 hrs, 2-3 times per week either under sedation (dexmedetomidine, 0.04 mg/kg) or awake. Neural activity and bladder pressure are recorded (Ripple Grapevine system) under various test conditions. The DRG or pudendal nerve are also electrically stimulated at 1 - 33 Hz to drive LUT activity. LUT activity has been recorded for 4 to 11 weeks in four animals. Microelectrode impedances have remained stable with, on average, 83 % ± 0.12 % of the total number of channels per test session

having values between 10 kΩ and 500 kΩ. Data analysis is still ongoing, though up to 18 bladder afferents have been identified in a given animal (correlation coefficient > 0.2) with a trend showing more bladder afferents emerging after the second week of implant. Some channels have consistently showed bladder afferent activity for up to 41 days and we have been able to track individual units for up to 37 days. Distension-evoked and pudendal stimulation-driven bladder emptying has been observed during which urethra flow afferents have been identified. Voiding during awake sessions was also observed. Our chronic setup allows us to track LUT afferents under varying bladder conditions and drive spinal circuits using electrical stimulation. We can monitor LUT activity in both sedated and awake conditions and are working on improvements in our chronic setup. From this work we hope to gain a better understanding of LUT neurophysiology, including during awake conditions, and evaluate the feasibility of such a set-up for closed-loop neuroprosthesis control.

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Poster

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Title: Transient receptor potential vanilloid 4 channels modulate Ca^{2+} signals in interstitial cells of Cajal at urothelial-lamina propria junction of rat pups.

Authors: *M. A. VIZZARD[1], M. T. NELSON[2], T. J. HEPPNER[2];
[1]Neurolog. Sci., [2]Pharmacol., Univ. Vermont Col. Med., Burlington, VT

Abstract: The urinary bladder wall contains interstitial cells of Cajal (ICCs) that may play a role in bladder function by modulating communication between nerve fibers and smooth muscle or acting as pacemakers. Transient receptor potential vanilloid 4 (TRPV4) channels allow cation influx and may be involved in sensing stretch or chemical irritation in urinary bladder. A layer of ICCs expressing platelet-derived growth factor receptor (PDGFR)-alpha-immunoreactivity and exhibiting a branching, stellate morphology was identified at the interface of the urothelium and lamina propria. In this study we investigated the role of TRPV4 channels to modulate Ca^{2+} signals in urothelial-lamina propria junction ICCs. Urothelium was removed from rat pups (< 3 weeks old), cut into strips, and mounted on sylgard blocks. The urothelial strips were loaded with a Ca^{2+} fluorescent dye (Fluo-2 AM leak resistant) for 90 minutes (37^{o} C) to measure Ca^{2+} events. The sylgard blocks with attached urothelial strips were placed in a special imaging chamber and superfused with PSS (37^{o} C). Ca^{2+} events were recorded for a period of 60 seconds in control and after drug treatment. Analysis of Ca^{2+} events was performed post hoc using software developed by Dr. Adrian Bonev (UVM). A region of interest (ROI) was placed over the cell and changes in Ca^{2+} fluorescence within the ROI were compared to baseline (F/F_o). ICCs, located between the basal cell layer and the uppermost level of the lamina propria exhibited slow changes in Ca^{2+} that sometimes appeared to travel to neighboring cells through branched processes. To test for the presence of functional TRPV4 channels the TRPV4 agonist GSK1016790 (100 nM) was applied to the urothelium through the superfusing PSS. In paired recordings, GSK1016790 increased the number of Ca^{2+} events from 127 to 295 (132% increase) (n = 100 cells, 5 paired fields; 15-20 cells/field) whereas the TRPV4 antagonist GSK2193874 (1 μM) decreased the number of Ca^{2+} events from 131 to 94 (38.2% decrease) (n = 94 cells, 6 paired fields; 11-25 cells/field). The amplitude and duration of Ca^{2+} events were not significantly changed by the TRPV4 agonist (control, 1.6 ± 0.07 F/F_o, 2.5 ± 0.25 sec; GSK1016790, 1.5 ± 0.03 F/F_o, 2.3 ± 0.19 sec, n = 35 cells); however the antagonist did significantly decrease the amplitude, but not the duration of Ca^{2+} events (control, 2.1 ± 0.09 F/F_o, 1.8 ± 0.07 sec; GSK2193874, 1.8 ± 0.07 F/F_o*, 3.2 ± 0.19 sec, n = 57 cells, mean ± S.E.M., *$p \leq 0.05$), in paired recordings, respectively. These findings suggest that TRPV4 channels modulate Ca^{2+} signals in this population of ICCs and may contribute to ICC activity and bladder function.

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Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Topic: F.07. Autonomic Regulation

Support: R21NS086413

Title: Connectome and putative function of MET-positive neurons in the vagal motor complex

Authors: *A. K. KAMITAKAHARA[1], H.-H. WU[1,2], P. LEVITT[1,2];
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Abstract: Children with Autism Spectrum Disorder (ASD) are three times more likely to have associated gastrointestinal disturbances (GID), however the biological mechanisms contributing to this overrepresentation are unknown. One genetic commonality for both ASD and GID is a promoter variant in the human *MET* receptor tyrosine kinase (*MET*) gene that is significantly enriched in families of children with both ASD and co-occurring GID. The presence of this promoter variant results in reduced *MET* gene expression, which has been shown to critically impair a number of neurodevelopmental processes, including neocortical and enteric neuron dendrite growth, hippocampal synapse maturation, and spinal motor neuron survival. MET is expressed by a subset of developing brainstem autonomic neurons that innervate the GI tract. To determine whether MET signaling impacts the establishment of vagal inputs to the GI tract, we characterized the subpopulation of neurons that express MET by projection target, and assessed MET function through conditional deletion of the *Met* gene in developing motor neurons. Immunohistochemistry in Met^{EGFP} mice revealed that both EGFP and MET protein are expressed by a subset of neurons in the dorsal motor nucleus of the vagus (DMV), and by the vast majority of nucleus ambiguus (nA) neurons in the compact part of the nucleus beginning approximately on E11.5, shortly after these neurons are generated. Innervation targets of MET neurons in the brainstem vagal motor nuclei were determined through injection of the retrograde tracer, cholera toxin B, into sites along the GI tract. This revealed anatomically distinct subsets of MET neurons in the DMV, which segregate by projection target. MET expressing neurons rostral to the obex were located medially and projected to the stomach, whereas MET expressing neurons caudal to the obex were located laterally and projected to the cecum. In addition, the muscular layers of the esophagus of Met^{EGFP} mice contained EGFP+ axonal projections, likely arising from neurons in the nA. To test the neurodevelopmental functions of MET in vagal brainstem motor neurons, MET was conditionally deleted from developing motor neurons in $Isl1^{cre}; Met^{flox/flox}$ mice. On embryonic day 16.5, an approximate 50% reduction in the number of neurons in the nA was observed in $Isl1^{cre}; Met^{flox/flox}$ mice compared to control mice, suggesting that MET is required for formation of the nA. Ongoing analysis of the density of GI innervation by vagal MET

neurons in *Isl1^{cre}; Met$^{flox/flox}$* mice will advance understanding of how the reduced contribution of nA impacts autonomic innervation in the developing GI tract and give insight into increased GID risk in ASD.

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Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Topic: F.07. Autonomic Regulation

Support: JSPS Grant-in-Aid for Exploratory Research (Grant Number: 26670268)

Title: Detection of nausea in rats using the monitoring of facial expression.

Authors: *K. YAMAMOTO, S. TATSUTANI, T. ISHIDA;
Osaka Univ., Suita, Japan

Abstract: Patients receiving cancer chemotherapy experience nausea and vomiting. They are not life-threatening symptoms, but their insufficient control reduces the patient's quality of life and become a risk factor for refusal to undergo further therapy. To identify preventing methods of nausea and vomiting in preclinical study, objective evaluation of these symptoms in experimental animals is required. Unlike vomiting, nausea is defined as a subjective feeling described as recognition of the need to vomit; thus, determination of the severity of nausea in experimental animals is considered to be difficult. However, since we observed that rats make a grimace after administration of cancer chemotherapeutic agents, cisplatin, we hypothesized that changes of facial expression are used as a method to detect nausea. In this study, we examined to detect the changes of facial expression of rats after administration of cisplatin. Furthermore, we investigated the effect of anti-emetic drugs on the prevention of cisplatin-induced changes of facial expression in rats.
Male Wistar/ST rats were housed in individual cages with free access to food and tap water, and their facial expression was continuously recorded by infrared video camera. On the day of the experiment, rats received cisplatin (0 or 3 mg/kg, i.p.) with or without a daily injection of a 5-HT_3 receptor antagonist (granisetron: 0.1 mg/kg, i.p.) or a neurokinin NK_1 receptor antagonist (fosaprepitant: 2mg/kg, i.p.), and their eye-opening degree (L/W ratio: the ratio between longitudinal and wide length of eye) was analyzed by the recorded video image.
Intraperitoneal injection of cisplatin significantly induced decrease of L/W ratio after 6 hours of the injection of cisplatin and the decrease continued for 2 days. The acute phase (day 1), but not

the delayed phase (day 2), of decrease of L/W ratio was inhibited by treatment of granisetron; however, fosaprepitant abolished both phases of changes of L/W ratio.
The profiles of facial expression are similar to clinical evidence of cisplatin-induced nausea in humans. These findings indicated that the changes of facial expression has the potential to detect nausea in experimental animals.

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Poster

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Topic: F.07. Autonomic Regulation

Title: Pudendal nerve stimulation elicits oscillations in vaginal blood flow

Authors: *I. C. RICE[1,2], L. L. ZIMMERMAN[1,2], S. E. ROSS[1,2], M. B. BERGER[3], T. M. BRUNS[1,2];
[1]Biomed. Engin., [2]Biointerfaces Inst., Univ. of Michigan, Ann Arbor, MI; [3]Obstetrics and Gynecology, Univ. of Michigan Hlth. Syst., Ann Arbor, MI

Abstract: Female sexual dysfunction is a widespread clinical problem that affects over 25% of women. The mechanisms of female arousal are not well understood, and there are few treatment options. Neuromodulation for bladder and bowel dysfunction has shown the potential for improving symptoms of sexual dysfunction, supporting the potential of a targeted nerve stimulation approach. The purpose of this study is to investigate the use of pudendal nerve stimulation in female rats as a means for understanding and developing potential treatments for female sexual dysfunction.
Under ketamine anesthesia, platinum-wire hook electrodes were placed on the pudendal nerve and secured with Kwik-Cast. Stimulation was performed with varying frequencies (1-40 Hz) and amplitudes (0.5-3 V). Stimulation was delivered for either 1-2 minute periods with 1-10 minute breaks, or for 20-30 minute periods with 20-30 minute breaks. Changes in vaginal blood flow, a standard proxy for arousal, were measured with a laser Doppler flowmetry probe inserted into the vagina and angled against the anterior wall. Vaginal luminal diameter (VLD) was measured as a secondary indication of arousal.
5-10 Hz pudendal stimulation often resulted in large increases (2-10x) in vaginal blood perfusion. Low frequency oscillations (0.01-0.9 cycles per second) were observed and maintained for up to 10 minutes following stimulation. During experiments where stimulation was delivered for shorter periods (1-2 minutes) and a response to stimulation was noted, 11-19

stimulation periods (over 1-2.5 hours) were necessary before an oscillatory blood flow response was observed. When the stimulation time was increased to 30 minutes, an oscillatory response was usually seen after either the first or second stimulation period. Concurrent with the oscillatory blood flow response, a VLD increase (1.3-1.8x) was observed in several of the experiments, as a result of both short and long stimulation periods.
To our knowledge, this is the first time that slow oscillations in vaginal blood flow due to peripheral nerve stimulation have been reported. Our study supports pudendal stimulation as a potential method for inducing increases in vaginal blood flow. The secondary indication of VLD increase further indicates that the oscillatory increases in vaginal blood flow may be an arousal response. Implementation of this technique could contribute to a treatment for female sexual arousal dysfunction by improving vaginal blood flow.

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Poster

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Title: EMG analysis of external urethral sphincter in awake spinal cord injured rats during urodynamic assessment

Authors: *M. P. SCHNEIDER, A. K. ENGMANN, T. M. KESSLER, M. E. SCHWAB; Univ. of Zürich, Zürich, Switzerland

Abstract: We recently developed a urodynamic set-up allowing repetitive, long term urodynamic measurements of both bladder and external urethral sphincter function in the same rat under fully awake conditions (Schneider MP et al., BJU International 2015). We now

assessed the development of lower urinary tract dysfunction as a consequence of large spinal cord injuries (SCI) in adult rats over 6 weeks.
A urodynamic catheter into the bladder and external urethral sphincter electromyography (EMG) electrodes were implanted into female Lewis rats. Two weeks after implantation, baseline urodynamic investigation showed a normal, fast, large volume voiding response to bladder filling. For the analysis of external urethral sphincter EMG activity profiles, fast Fourier transformation was used to generate heat plots with power per frequency and time. In intact rats, the external urethral sphincter showed strong peak activity in the high frequency range (21-500Hz, representing striated muscle activity) at the high filling state immediately before voiding, as well as after voiding. High frequency activity was low during micturition. In contrast, slow wave bursting activity (3-20Hz, representing smooth muscle activity) was prominent during voiding.
Spinal cord injured rats underwent a microsurgical subtotal transection at the level of T9 which destroyed the grey, dorsal white and dorsolateral white matter completely and bilaterally, sparing only 10-20% of the ventral white matter. 14 of these 15 rats developed detrusor sphincter dyssynergia (DSD) characterized by high tonic high frequency sphincter EMG activity during the voiding phase, which resulted in dripping and interrupted release and very prolonged voiding phases. These characteristics are closely analogous to the one's seen in severely spinal cord injured patients. Studies are on-going to unravel the underlying lesion induced neuronal changes in the spinal cord, the primary afferent fibers and the remaining descending tracts.

Disclosures: M.P. Schneider: None. **A.K. Engmann:** None. **T.M. Kessler:** None. **M.E. Schwab:** None.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 449.15/GGG12

Topic: F.07. Autonomic Regulation

Title: Evaluating sexual arousal in a female rat model with tibial nerve stimulation

Authors: *L. ZIMMERMAN[1,2], I. C. RICE[1,2], M. B. BERGER[3], T. M. BRUNS[1,2]; [1]Biomed. Engin., [2]Biointerfaces Inst., Univ. of Michigan, Ann Arbor, MI; [3]Obstetrics and Gynecology, Univ. of Michigan Hlth. Syst., Ann Arbor, MI

Abstract: The aim of this research is to determine if tibial nerve stimulation can have an effect on sexual arousal, using a female rat model. Currently, there are limited treatments for women suffering from female sexual dysfunction (FSD), a condition which can include deficits in desire,

arousal, and lubrication. In clinical trials involving percutaneous tibial nerve stimulation (PTNS) for bladder voiding dysfunction, female patients sometimes note an additional effect of positive impact on sexual function. However, there have been no studies attempting tibial nerve stimulation specifically for sexual function, separate from bladder function research. In ketamine-anesthetized female rats, we isolated and stimulated the tibial nerve on one side with platinum-iridium wire hooks. The stimulation frequency was between 10-20 Hz with an amplitude that was 2-5 times the threshold for causing a distal muscle response. Stimulation was delivered for 30 minute periods, with 20-30 minute breaks, across 3-4 hours. Vaginal blood perfusion was measured with laser Doppler flowmetry as the primary proxy for arousal. Vaginal luminal diameter was measured as a secondary indication. After thirty-minute periods of stimulation on the tibial nerve, large increases in blood perfusion (up to 10x average pre-stimulation amplitudes) with low frequency oscillations (0.1-0.6 cycles per second) were observed and maintained for several minutes. The oscillations are similar to those reported in clinical studies of vaginal blood flow during sexual arousal. Large increases in vaginal luminal diameter (up to 3.4x pre-stimulation diameter) were also observed, indicating that the modulations in blood perfusion may be due to arousal rather than pelvic floor contractions. These preliminary results suggest that tibial nerve stimulation may contribute to a therapeutic approach for a genital arousal component of FSD.

Disclosures: L. Zimmerman: None. **I.C. Rice:** None. **M.B. Berger:** None. **T.M. Bruns:** None.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Program#/Poster#: 449.16/GGG13

Topic: F.07. Autonomic Regulation

Support: Research Grant, 2014-2015 from Ministry of Defense

Title: High-salt intake decreases intestinal Na^+/K^+-ATPase activity in normotensive rats but not in hypertensive Dahl salt-sensitive rats

Authors: *M. TANDAI-HIRUMA, T. KEMURIYAMA, Y. NISHIDA;
Physiol., Natl. Def Med. Col., Saitama, Japan

Abstract: The specific mechanisms by which high-salt cause salt-sensitive hypertension have been clarifying. Oral high-salt is first sensed by the brain to enhance the production of endogenous ouabain followed by chronical enhancement of the secretion of cardiotonic steroids from adrenal. Those affect target peripheral organs by both inhibiting the pump activity of

Na^+/K^+-ATPase (NKA) and activating the intracellular signaling pathway via NKA. In the proximal renal tubules (PRT) of normotensive Sprague-Dawley (SD) rats, those enhance the endocytosis of basolateral NKA and luminal Na^+/H^+ exchanger 3 to stimulate natriuretic response, which is suppressed in hypertensive Dahl salt-sensitive (DSS) rats. On the other hand, we observed that 4 weeks of high-salt intake increased the intestinal secretion of Na^+, Cl^-, and water in SD but not in hypertensive DSS rats. Since the mechanism of an intestinal ion transport has many similarities with PRT, the aim of this study is to elucidate whether high-salt intake change intestinal NKA activity in hypertensive DSS rats. Male DSS and SD rats were divided into two groups; one fed on a high-salt diet (DSSH and SDH), and the other fed on a regular diet (DSSR and SDR) for 4 weeks. The intestinal adsorption-secretion balance was accessed by examination of feces materials. Feces were scored from 1 to 4 based on color, texture, and amount. Normal feces, exceptionally loose feces, loose feces, and loose & partially watery feces were given an Index 1, 2, 3, and 4, respectively. Next, to compare the effect of high-salt diet on NKA activity between DSS and SD rats, the intestinal mucosa-submucosal preparations from each group were mounted on the Ussing chamber to measure short-circuit current (I_{sc}) induced by nystatin to permeabilize mucosal side in the presence or absence of mucosal Na^+. Nystatin-induced I_{sc} in the presence of mucosal Na^+ represents basolateral Na^+ outward current driven by NKA and we ascertained that this current almost all varnished in the absence of mucosal Na^+. Feces Index was significantly higher in SDH than that in SDR, while no significant difference was found between DSSH and DSSR, indicating that high-salt diet activate water secretion in SD but not in DSS rats. Furthermore, high-salt diet significantly decreased nystatin-induced I_{sc} in SD (129.4 ± 6.9 for SDH versus 179.7 ± 7.3 $\mu A/cm^2$ for SDR) but not in Dahl (208.7 ± 10.8 for DSSH versus 198.4 ± 9.6 $\mu A/cm^2$ for DSSR) rats indicating that high-salt diet decreases NKA activity in SDH but not in DSSH. These results imply that unchanged basolateral NKA activity even on high-salt diet is one of the causes of the inconsistent intestinal ion transport in hypertensive DSS rats.

Disclosures: M. Tandai-Hiruma: None. **T. Kemuriyama:** None. **Y. Nishida:** None.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Program#/Poster#: 449.17/GGG14

Topic: F.07. Autonomic Regulation

Support: NIH R37 DK5482

NIH R01 DK057284

Title: Estrus-cycle dependent variations regulate sensitivity of rat urinary bladder urothelial cells

Authors: *A. S. WOLF-JOHNSTON, F. A. KULLMANN, L. A. BIRDER;
Sch. of Med., Univ. Pittsburgh, Pittsburgh, PA

Abstract: Introduction: A growing body of evidence suggests that symptoms in many chronic pelvic pain syndromes may be influenced by fluctuation in hormones- in particular estrogen (E2). Studies have shown that urinary bladder epithelial (urothelial-UT) cells, which line the bladder lumen, express E2-receptors. In addition, rapid or non-genomic effects of E2 may play a role in release of mediators and/or activation of signaling pathways. The goal of this study was to examine the mechanisms by which E2 alters urothelial sensory functions.
Methods: UT cells (UTCs) were cultured from rat urinary bladders using previously described methods in accordance with approval by the University of Pittsburgh Institutional Animal Use and Care Committee. Phosphorylated (p-) p38 expression was examined in whole cell lysates isolated from UTCs or bladder mucosal tissue and subjected to western blotting using a standard protocol. ATP release was examined by collecting effluent following application of chemical stimuli (TRPV1 agonist capsaicin- 10 μM; βE2-100 nM) with or without pretreatment with E2 antagonists. Statistical significance was considered with $p < 0.05$.
Results: Capsaicin evoked ATP was potentiated in the presence of 100nM b-E2 and release was prevented with either Tamoxifen (1μm) or ICI-182,780 (3μm), two estrogenic antagonists. We also find β-E2 increased the expression of (activated) p38 MAPK. The degree to which pp38MAPK expression was increased after β-E2 exposure was linked with estrus cycle. The increase in pp38MAPK was increased in UTC collected from rats in estrus, but not from rats in diestrus or proestrus.
Conclusions: Our preliminary findings suggest that estrogens (via beta-receptors) can acutely alter responsiveness of bladder epithelial cells to chemical stimuli. Studies have shown that estrus cycle dependent fluctuations can have profound effects on algogenic mediators and on pain thresholds. Thus, our findings of augmented levels of stress-kinase expression seen in rats in estrus suggest that variations in gonadal hormones may contribute to pain fluctuations in female patients with visceral pain disorders.

Disclosures: A.S. Wolf-Johnston: None. **F.A. Kullmann:** None. **L.A. Birder:** None.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Topic: F.07. Autonomic Regulation

Support: NIH F32 DK098904

R01 NS050514

Title: A novel method to non-invasively determine the post-void residual in continuous serial cystometrograms

Authors: *Z. C. DANZIGER, W. M. GRILL;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: A serial cystometrogram (SCMG) is the process of continuously filling the bladder to induce multiple voiding cycles. This technique is commonly used to understand better the behavior of the lower urinary tract in disease models and to assess the urodynamic response of animals to treatments. While multiple informative urodynamic parameters can be measured during a SCMG, there is no easy, non-invasive way to measure the post-void residual volume (PVR). Currently, obtaining PVR from a SCMG requires either ignoring the unknown ureter flow into the bladder, which systematically underestimates PVR, or removing the PVR after each void to measure it directly, which is not physiological and disrupts the system being studied. Therefore, standard urodynamic parameters that require PVR for their calculation, such as voiding efficiency and bladder capacity, are not readily available.
We developed a procedure to estimate accurately the PVR for each void in a SCMG without the need to remove PVR from the bladder during the experiment. The procedure is derived from a recursive mass-balance equation on the bladder, and uses available measurements to estimate the ureter contribution to bladder volume and calculate PVR. We validated the accuracy of the procedure with numerical simulations and with in vivo SCMG experiments in urethane-anesthetized rats. The estimation procedure is at least as accurate as withdrawing PVR from the bladder for direct measurements. We also assessed the physiological effects of measuring PVR after each void in a SCMGs in an *in vivo* rat model of acetic acid-induced cystitis, and found that it results in an increase in the variability of bladder capacity as compared to our non-invasive procedure. The novel estimation procedure provides a way to calculate PVR in SCMGs that is more accurate than currently available methods, preserves the natural physiological conditions in the lower urinary tract by not removing PVR after each void, and does not irritate the bladder through repeated invasive measurements. Further, our estimation procedure requires no experimental setup or equipment beyond that of a typical SCMG study. The estimation procedure is a very useful new tool for basic science research in *in vivo* animal studies of lower urinary tract function.

Disclosures: Z.C. Danziger: None. **W.M. Grill:** None.

Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Program#/Poster#: 449.19/GGG16

Topic: F.07. Autonomic Regulation

Support: Craig. H Neilson Foundation (#314980)

Title: Kalman filter decoding of bladder pressure from dorsal root ganglia activity

Authors: *A. OUYANG, S. E. ROSS, T. M. BRUNS;
Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

Abstract: A closed-loop bladder neural prosthesis provides the potential to alleviate bladder dysfunction problems by delivering neuromodulatory stimulation at desired pressures to control bladder excitation and relaxation. Previous studies have shown that bladder sensory neural signals can be recorded from sacral-level dorsal root ganglia (DRG). These neural signals can be used to infer information about the bladder pressure. In this study, we evaluate the effectiveness of a Kalman filter estimator as an approach to predict bladder conditions, including bladder pressure, pressure rate and baseline pressure, using sacral-level DRG neural signals as input. We acquired neural recordings from S1 and S2 DRG microelectrodes during acute alpha-chloralose anesthetized feline procedures. We also monitored bladder pressure while saline was infused at 2ml/min until the bladder was full. The performances of linear regression and a linear Kalman filter estimator were evaluated and compared. A Kalman filter was selected due to its ability to reduce statistical noise from the imperfect measurements and potentially improve upon linear regression. For each experiment, in MATLAB, a training model was developed using unsorted neural data from an early bladder fill (training). This model was then applied to neural data from a later bladder fill to estimate the pressure (testing). Across nine training bladder fills, a Kalman filter (4.0 cmH_2O root-mean-squared error) had a better fit than linear regression (9.0 cmH_2O root-mean-squared error). The training model was applied to a test bladder fill data set in four cases, and again led to a lower estimate (Kalman: 10.6 cmH_2O; Linear: 12.8 cmH_2O). Training and testing of the Kalman filter model only added a few programming steps, which did not increase the computation time. Data analysis is on-going. These results suggest the potential of a Kalman filter to be used in estimating bladder pressure for a closed-loop bladder neuroprosthesis based on DRG activity.

Disclosures: A. Ouyang: None. **S.E. Ross:** None. **T.M. Bruns:** None.

Poster

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Topic: F.07. Autonomic Regulation

Support: The Ministry of Education, Culture, Sports, Science and Technology of Japan #26870207

Title: Central inhibition of initiation of swallowing by systemic administration of diazepam and baclofen in anaesthetized rats

Authors: *T. TSUJIMURA[1], S. SAKAI[1], T. SUZUKI[1], K. TSUJI[1], J. MAGARA[1], B. J. CANNING[2], M. INOUE[1];
[1]Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata City, Japan; [2]Johns Hopkins Asthma and Allergy Ctr., Baltimore, MD

Abstract: Purpose: We hypothesized that glutamate receptor antagonists and GABA receptor ligands, which are used clinically for multiple disorders, may modify swallowing function. The aim of present study was to investigate the effects of memantine, diazepam, baclofen and dextromethorphan on swallowing evoked by multiple stimuli delivered directly to the upper gastrointestinal tract or by electrical stimulation of the superior laryngeal nerve (SLN). Materials and Methods: Urethane-anesthetized male Sprague Dawley rats were used. Swallows were evoked by upper airway (UA)/pharyngeal distension, punctate mechanical stimulation using a von Frey filament, capsaicin or distilled water (DW) applied topically to the vocal folds, and electrical stimulation of a SLN in anesthetized rats and were documented by recording electromyographic activation of the suprahyoid and thyrohyoid muscles and by visualizing laryngeal elevation. The effects of intraperitoneal or topical administration of each drug on swallowing function was studied. Results & Discussion: Systemic administration of diazepam and baclofen, but not memantine or dextromethorphan, inhibited swallowing evoked by mechanical, chemical and electrical stimulation. Both benzodiazepines and $GABA_A$ receptor antagonists diminished the inhibitory effects of diazepam while a $GABA_B$ receptor antagonist diminished the effects of baclofen. Topical applied diazepam or baclofen was without effect on swallowing. These data indicate that diazepam and baclofen act centrally to inhibit swallowing in anesthetized rats.

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Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Topic: F.07. Autonomic Regulation

Support: UNAM-DGAPA-PAPIIT IN223714

Title: Intratesticular administration of p-chloroanfetamine (pca) alters sperm quality.

Authors: J. A. DIAZ-RAMOS[1], C. A. DON-LOPEZ[1], A. L. RODRÍGUEZ-GUTIERREZ[1], M. FLORES-FLORES[1], *R. DOMINGUEZ[1], M. E. AYALA-ESCOBAR[1], A. ARAGON-MARTINEZ[2];
[1]FES Zaragoza UNAM, Mexico City DF, Mexico; [2]Inst. Tecnologico del Altiplano de Tlaxcala, Tlaxcala, Mexico

Abstract: The functions of the testis, spermatogenesis and steroidogenesis, are regulated by the gonadotropins and other factors, as serotonin. In testis, Leydig cells synthesize serotonin, which in turn acts as an autocrine regulator of testosterone secretion. Amphetamine derivate, pCA, has neurotoxic effects on the serotonergic system. The chronic administration of pCA to prepubertal male rats results in lower concentrations of serotonin in the hypothalamus and diminution in the spermatognenesis. In addition, a single injection of pCA to prepubertal male rats decreases testosterone secretion 72 hours after the treatment and this changes in testosterone did not correlate with modifications in LH secretion, suggesting a direct effects of the drug on the testis. Therefore, we analyzed the effects of pCA injection on the sperm quality.
Thirty-day-old male rats of the CIIZ-V strain from our own breeding stock were allocated at random to one of the following groups: animals injected vehicle or PCA (0.03mg), (0.06mg) or (0.12mg) dissolved in 20 microL of 0.1% of NaCl in both testis. An untouched control group was included. The rats were autopsied at 65 days of age. The vas deferens were dissected and its content obtained and placed in Hanks' balanced solution, incubated for 30 m. The mitochondrial activity and DNA fragmentation were evaluated by flow cytometry. The number of sperm and of abnormalities were measured in a Newbauer's chamber.
We did not observe differences between the control and vehicle injected rats. The injection of 0.06 or 0.12 mg of pCA resulted in lower weight of the testis than in vehicle injected group (0.06 mg: 1.50+/-0.027 gr; 0.12mg: 1.51+/-0.05 gr vs. 1.60+/-0.03gr, $p<0.004$ analysis of variance). The percentage of normal spermatozoa was lower than in vehicle group (0.06 mg: 77.19; 0.12mg: 65.49 vs. 96.21 $p<0.05$). In pCA injected group the number SYBR positive cells measured by flow cytometry was lower than in control (0.03mg: 16.67+/-2.86; and 0.06mg: 14.80+/-3.27 vs. 32.91+/-5.51), suggesting a decrease in normal condensed DNA in spermatozoa. Mitochondrial activity in pCA injected animals was similar to control.

Present results suggest that serotonin participates in the modulation spermatogenesis, both in sperm quality and quantity.

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Poster

449. Gastrointestinal: Urinary and Renal Regulation

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Title: plasticity in trpv4 expression and function in micturition reflex pathways during postnatal rat development

Authors: ***B. M. GIRARD**, S. MALLEY, M. VIZZARD;
Anat. and Neurobio., Univ. of Vermont Dept. of Neurolog. Sci., Burlington, VT

Abstract: The storage and periodic elimination of urine exhibit marked changes during prenatal and postnatal development but the mechanisms underlying these changes are largely unknown. Prior to maturation of the nervous system, urine is presumably eliminated from the urinary bladder by non-neural mechanisms. As the central nervous system continues to mature during the postnatal period, reflex voiding is brought under voluntary control involving higher brain centers. Transient receptor potential cation channel vanilloid family member 4 (TRPV4) is expressed in a variety of tissues/cells, including the urinary bladder and sensory neurons, but developmental regulation of expression has not been studied. We demonstrate developmental expression of TRPV4 in bladder sensory neurons (Fastblue (FB) labeled) and urothelium+suburothelium from postnatal and adult rat. TRPV4 channels are strong candidates for mechanosensors in the urinary bladder. In contrast, we propose a novel paradigm whereby the TRPV4/Ca^{2+} signaling complex acts as a brake to the mature micturition reflex during the early postnatal period. Using Q-PCR and immunohistochemistry and confocal imaging, we demonstrate expression of TRPV4 transcripts in postnatal (P) rats (P3, P5, P10, P12, P21, Adult) in urothelium+suburothelium and dorsal root ganglia (DRG). TRPV4-immunoreactivity is

expressed in whole mounts and sections (7 μm) of urothelium and in FB-positive sensory neurons in lumbosacral DRG retrogradely labeled from the urinary bladder in postnatal and adult rats. We performed open cystometry in conscious postnatal (P5-P12) rat pups with intravesical infusion of saline before and after intravesical infusion of TRPV4 antagonist, HC067047 (1 μM). Before TRPV4 blockade, P5-P12 rat pups only voided with perigenital stimulation. After TRPV4 blockade with HC067047, P5-P12 rat pups void with intravesical infusion of saline in absence of perigenital stimulation. These studies demonstrate that intravesical instillation of the TRPV4 antagonist, HC067047, turns on a mature micturition reflex (distention-induced) in early postnatal rat pups. The TRPV4 antagonist is highly lipophilic; thus, intravesical infusion may affect several tissue/cell types in the urinary bladder, including the urothelium, interstitial cells of Cajal, sensory nerves and detrusor smooth muscle. Understanding the mechanisms that underlie the transition of the neonatal bladder to a mature urinary bladder with efficient storage and elimination is important for understanding childhood and adult voiding dysfunction and reemergence of immature voiding reflexes after injury.

Disclosures: **B.M. Girard:** None. **S. Malley:** None. **M. Vizzard:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.01/GGG20

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS085757

Title: Polysomnographic characterization of nocturnal sleep in Cynomolgus macaques

Authors: *A. V. GOONAWARDENA[1], M. DI ZAMBOTTI[2], A. R. WILLOUGHBY[2], C. GLAVIS-BLOOM[1], I. M. COLRAIN[2], T. L. WALLACE[1], T. S. KILDUFF[1];
[1]Ctr. for Neurosci., [2]Ctr. for Hlth. Sci., SRI Intl., Menlo Park, CA

Abstract: Macaques are a diurnal species that exhibit consolidated night-time sleep with evidence of NREM and REM sleep similar to humans. Since many psychiatric medications can affect sleep, we sought to determine whether Cynomolgus macaques are a useful species in which to conduct polysomnographic sleep/wake studies with translational potential to humans. Adult, male Cynomolgus macaques (n=4) were implanted with telemetry transmitters for electroencephalography (EEG) and electromyography (EMG) and 12-h recordings during the dark period were initiated prior to light offset. EEG activity during wake, NREM and REM was scored in 30s epochs using the guidelines of the American Academy of Sleep Medicine (2007)

for scoring human sleep records and was quantified across polysomnographic measures and by spectral frequencies. Vehicle-treated macaques showed a sleep onset latency (SOL) of 12.6±2.8 min after light offset, 120.8±11.7 min of Wakefulness after initial sleep onset (WASO), and a Sleep Efficiency (SE) of 81.0±1.9%. Of the total sleep time (TST), most time was spent in N2 and N3 NREM stages (84%). The mean number of NREM/REM cycles in the vehicle-treated monkeys was 11±0.7 per night. Caffeine (10mg/kg, im) was administered immediately prior to the dark phase to induce wakefulness at a time of day when sleep pressure was high. Consistent with the stimulant properties of the drug, caffeine prolonged SOL compared to controls and decreased SE greatly as TST and the amount of both REM and NREM sleep were reduced. In addition, spectral power within the conventional frequency bands was calculated for each vigilance state, and, as expected, relative delta (1-4Hz) power was higher during NREM than both REM sleep and Wake in vehicle-treated macaques. Similarly, the relative theta (4-8Hz) power was higher during REM than NREM sleep and wakefulness. Furthermore, alpha (8-12Hz), beta (15-30Hz) and gamma (30-100Hz) power during Wake were greater than during NREM sleep. Caffeine-treated animals showed a significant decrease in delta and increases in alpha and sigma during NREM sleep compared to controls. In summary, the general features of human sleep are present in macaques, however, the NREM/REM cycle is shorter resulting in more cycles per night. Macaques respond to caffeine with longer SOL, less NREM and REM sleep, "lighter" N3 sleep and increased REM latency, similar to reports in humans. Together, these results indicate that Cynomolgus macaques show sleep characteristics consistent with humans under both normal conditions and after treatment with a psychostimulant.

Disclosures: A.V. Goonawardena: None. **M. di Zambotti:** None. **A.R. Willoughby:** None. **C. Glavis-Bloom:** None. **I.M. Colrain:** None. **T.L. Wallace:** None. **T.S. Kilduff:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Program#/Poster#: 450.02/GGG21

Topic: F.08. Biological Rhythms and Sleep

Title: Statistical source separation of rhythmic LFP patterns during sharp wave ripples in the macaque hippocampus

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

Manchester, United Kingdom; [4]Empirical Inference, Max Planck Inst. for Intelligent Systems, Tuebingen, Germany

Abstract: Sharp wave-ripples (SWR), episodes in the hippocampal CA1 local field potential (LFP) combining a low-frequency deflection (sharp wave) and a high-frequency oscillation (ripple), are thought to mediate memory consolidation. These events are paradigmatic episodes of the interaction between neuronal ensembles across distinct substructures of the hippocampal formation. However, the detailed neuronal ensemble mechanisms underlying this phenomenon remain largely unknown. This question arises partly due to inherent difficulties in inferring network-level dynamics from neuronal population measurements such as LFP. To address this question, we analysed *in-vivo* intracortical recordings of the CA1 of macaque monkeys. We devised a statistical source separation technique in order to disentangle the spatio-temporal signature of multichannel LFP in peri-SWR time windows of 1s. The first results of our study revealed that SWR complexes in CA1 can be approximated by a linear combination of four main oscillatory components with distinct spectral signatures. We found that SW (5.4-14.2 Hz) and gamma (31.7-68.1 Hz) components are expressed by *stratum radiatum*, while ripple (96.9-125.5 Hz) and supra-ripple (188.3-199.4 Hz, 95% confidence intervals) oscillations originate in stratum pyramidale. We then devised a model of the macaque's CA3-CA1 network. The network consists of two layers, each with 200 pyramidal-neuron and 20 peri-somatic interneuron models of two compartments. The model was able to predict a large number of features of *in-vivo* SW episodes. In particular, we found that SW (5.6-7.5 Hz), gamma (23.2-34.0 Hz), ripple (155.8-167.4 Hz) and supra-ripple (174.4-194.7 Hz, 95% confidence intervals) are also the main oscillatory components of modelled SWR. Our model suggests that SW and gamma components arise from CA3 bursting in *stratum radiatum*, while ripple oscillations originate from local interactions between pyramidal cells and interneurons. Notably, CA1 interneurons, also entrained by CA3-gamma oscillations, are responsible for the high-frequency component of the LFP activity, thus establishing the population signature of supra-ripple LFP during SWR. Our experiments suggest that SW, gamma, ripple and supra-ripple rhythms are specific markers of the phenomena occurring in neuronal activity during SWR that can be automatically extracted from LFP data with our approach. Finally, this approach establishes a relationship between neuronal activity over meso- and microscopic scales that can be used to investigate network properties such as excitation-inhibition balance without resorting to single unit analysis.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: NC123340.1

Title: The expression of dreams: Emotional facial expressions during REM sleep associated to dream mentation in depressed and healthy women

Authors: *A. P. RIVERA[1,3], I. RAMÍREZ SALADO[2], E. LÓPEZ RUIZ[2], J. GONZÁLEZ OLVERA[3], F. AYALA GUERRERO[3], J. PIÑA[2], A. SILVA CABALLERO[2], B. REYES ARANGUREN[2], D. CASTRO NIETO[2], A. JIMÉNEZ ANGUIANO[3];
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Abstract: Introduction: There is a strong association between facial muscle contractions (FMC) and emotional dream mentation (EDM) of awakenings from REM sleep compared to those of NREM sleep, thus being a reliable biomarker of emotional modality (valence) and intensity (arousal) of dreams. Notably, several alterations of phasic and tonic components of REM sleep are present in patients with Major Depressive Disorder (MDD). Yet, the possible association between FMC and EDM in patients with MDD remained to be explored.
Objective: To analyze FMC during REM sleep in female patients with MDD and its association with EDM as compared with those of female controls.
Method: One 8h standard sleep PSG recording was obtained from 10 healthy women and from 10 women with MDD without treatment. Facial EMG recordings were obtained from right and left Corrugator (Corr) and Zygomatic Major (Zyg) muscles. Experimental awakenings exploring EDM were performed during *in vivo* REM sleep stages that lasted at least three minutes. Awakenings were determined by a FMC that lasted more than 100 ms and by the amplitude of any facial muscle that exceeded by 500% its background activity. Following sleep, FMC were quantified, mean voltage (μv) per muscle during REM sleep was obtained and differences between MDD and controls were statistically evaluated. EDM global scores were obtained through the Dream Content Questionnaire with five blind judges. Correlation coefficients were calculated for FMC and EDM global scores and differences between groups (control vs. MDD) were evaluated by Friedman and Post Hoc tests.
Results: Mean voltage of both Corr and Zyg was significantly abolished in MDD group. Mean frequency of Zyg was diminished in the MDD while Corr's mean frequency was higher compared to controls. EDM in MDD was more negative and with lower intensity (e.g. sadness) than in controls, in which emotions ranged from high negative (e.g. anger), high positive (e.g. joy) to low positive (e.g. calm), while low negative was significantly diminished or absent. In controls, high positive EDM was positively associated to FMC of the Zyg and high negative EDM correlated with FMC of the Corr, whereas in MDD only Corr activity showed an association to low negative EDM.
Conclusions: The present study confirmed that FMC and EDM during REM sleep are specifically modified by MDD. We suggest that the neurophysiologic mechanisms altered in depression, i.e. impairment of amygdala-prefrontal cortex connectivity may be modifying dream content towards lower negative levels, and thus the inhibition of facial muscle activity may be a

direct reflect of this alteration, serving as a specific affective and not somatic biomarker of depression.

Disclosures: **A.P. Rivera:** None. **I. Ramírez Salado:** None. **E. López Ruiz:** None. **J. González Olvera:** None. **F. Ayala Guerrero:** None. **J. Piña:** None. **A. Silva Caballero:** None. **B. Reyes Aranguren:** None. **D. Castro Nieto:** None. **A. Jiménez Anguiano:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

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Pilot award 8G12MD00760 to J.C.E.

Title: Sleep amount is a potential biomarker for predicting behavioral responses in a mouse model of PTSD

Authors: ***C. L. GRAY**, L. PINCKNEY, E. N. OLIVER, K. N. PAUL, J. C. EHLEN; Morehouse Sch. of Med., Atlanta, GA

Abstract: Sleep disorders are often comorbid with psychological disorders like post-traumatic stress disorder (PTSD). However, the relationship between sleep and the development and maintenance of the behavioral hallmarks that accompany these disorders are mostly unknown. Previously, we determined that increased sleep prior to social defeat predicted susceptibility. In the present study, we examined sleep following social defeat to determine the influence of sleep on stress-induced avoidance behavior. Our hypothesis is that post-defeat sleep is protective against stress-induced behavioral changes. We implanted C57Bl6/J mice with electroencephalography and electromyography electrodes, recorded baseline sleep and then subjected the implanted mice to 10 days of social defeat stress using a resident-intruder paradigm. Social avoidance testing and sleep recordings were then performed immediately after social defeat. We determined that resilient mice (mice that exhibit no decrease in social investigation post-defeat) displayed significantly increased sleep (NREM and REM) during the active period. Susceptible mice (which exhibit a significant decrease in social investigation post-defeat) exhibited no such increase in sleep. Resilient mice also had significantly decreased NREM delta power during their rest period post-defeat, indicating reduced sleep pressure in these mice. These results indicate that susceptible mice are unable to alter their post-defeat sleep,

whereas increased sleep post-defeat confers a protective effect. We are currently investigating the effects of sleep manipulation in this model to determine if our findings were due to sleep homeostasis or other mechanisms influencing sleep. Understanding how sleep affects behavior using our current model will allow us to develop treatments for psychological disorders such as PTSD that evidence changes in both behavior and sleep.

Disclosures: C.L. Gray: None. **L. Pinckney:** None. **E.N. Oliver:** None. **K.N. Paul:** None. **J.C. Ehlen:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: MH 60670

Department of Anesthesiology

Title: Sleep deprivation after trauma imparts resilience to post-traumatic stress disorder (PTSD)

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Abstract: Post-traumatic stress disorder (PTSD) is a debilitating depressive and anxiogenic mental state that develops following exposure to extremely traumatic events such as sexual assault and active military service in warfare. The Department of Veterans Affairs estimates that 22 United States military veterans commit suicide every day. The mechanism that determines those who are resistant or vulnerable to developing PTSD is currently unknown. Since alterations of the sleep cycle are an intrinsic component of aberrant mental health, they may serve as electrophysiological biomarkers predicting the development of PTSD symptomology. Because rapid eye movement (REM) sleep plays a critical role in memory consolidation and emotional regulation, we hypothesized REM sleep deprivation could exacerbate the PTSD phenotype. To investigate this, we placed male Sprague Dawley rats in a sleep-deprivation bin with multiple small platforms over water for 6 hours prior to (RD-B SPS; n=6) or after (RD-A SPS; n=6) implementing the single prolonged stress (SPS) rodent model for PTSD and measured fear memory extinction versus controls consisting of non-SPS (n=6) and SPS-treated rats never sleep

deprived (n=6). The SPS model establishes phenomenolocigal similarity (face validity), a corresponding theoretical framework (construct validity), and the ability to predict that a pharmacological agent will have the same amelioration on symptoms in both the animal model and in humans (predictive validity) (Yamamoto et al. 2009). SPS consisted of 2 hours of restraint, 20 minutes of forced swim in 24°C water, 15 minutes of recuperation, and exposure to ether until the loss of consciousness. Animals were then individually housed for 7 days of isolation and freezing levels on a fear-associated memory test were evaluated thereafter. The results of the study found the RD-A SPS condition to ameliorate the PTSD-like phenotype by impairing extinction recall (44.7% freezing) to below that of non-SPS controls (52.1% freezing). The PTSD-like phenotyope was highest in the SPS-only group (69.7% freezing), while the RD-B SPS condition did not significantly rescue animals from the effects of SPS (62.1% freezing). These data suggest that REM sleep-deprivation immediately following a traumatic event may prevent consolidation of the traumatic memory. A possible mechanism for adaptive fear-response may thus be an insomnia-response, that perhaps may be lacking or absent in those most susceptible to developing PTSD.

Disclosures: J. Dean: None. **J. Delorme:** None. **G. Poe:** None. **Y. Arias-Delphi:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: ONR

Title: Brief body-mind training improves sleep quality

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[3]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Research supports the claim that mindfulness meditation — practiced widely for the reduction of stress and promotion of health — exerts beneficial effects on physical and mental health, and cognitive performance. However, some studies also indicated that mindfulness meditation had moderate effects on anxiety, depression and pain, and low effects on stress/distress and mental health-related quality of life such as sleep quality. One major issue is that the methodological quality of meditation studies is relatively low, and some were cross-sectional studies using waitlist control and few were actively controlled longitudinal studies

using RCT design. In our series of RCT studies, we have been using one form of mindfulness meditation - the Integrative Body-Mind Training (IBMT) and relaxation training (RT) as an active control. We found that few hours of IBMT significantly improves attention, emotion, cognitive performance and reduces stress hormone cortisol. Several hours of IBMT changes brain structure associated with self-control ability. We hypothesize that better self-control will improve sleep quality. 225 healthy college students (age of 18-23 years old) without any training experience participated in the current study (115 in IBMT group, 110 in RT group). There were no differences in gender, age and education between two groups. The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep. It differentiates "poor" from "good" sleep quality by measuring seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month. We used PSQI to test whether 5 hours of IBMT (30 min per session for 10 sessions) could improve sleep quality in a college students population in comparison with the same amount of RT. Our results indicated that IBMT group showed significant improvements in global PSQI, sleep latency and sleep disturbances. RT group also showed improvements in global PSQI and sleep latency. Compared to RT, IBMT showed greater improvement in sleep latency. In sum, both IBMT and RT help improve sleep quality, but IBMT is also helpful for sleep disturbances..

Disclosures: **Y. Tang:** None. **X. Ding:** None. **R. Tang:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

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Title: Intensive practice during the day induces task-specific performance errors: the differential effects of nap and quiet rest

Authors: *A. B. NELSON[1], M. T. CHAN[2], J. LIN[2], P. PANDAY[2], J. BORKOWSKI[2], H. CHEN[2], M. GADALLA[2], B. O. THOMSON[2], G. TONONI[3], C. CIRELLI[3], M. F. GHILARDI[2]; [1]Dept. Physiology, Pharmacol. & Neurosci., [2]CUNY Med. Sch., New York, NY; [3]UW Madison, Madison, WI

Abstract: In a previous work (J Neurosci, 2015, 35: 4487), we found that when sleep-deprived subjects practiced a task for a prolonged time, their performance was more negatively affected in

tests that shared neural bases with the original practice than in other tasks. The increased rate of these errors became evident only after seventeen hours awake and worsened thereafter. These and other results in sleep-deprived animals suggest that such performance errors might be related to the occurrence of local sleep during wake. Here we postulate that local sleep is task-specific, is present without sleep deprivation, is induced by intensive training and reversed by a nap. Sixteen subjects were tested on two separate days from 8 am to 8 pm. One day they performed a task involving significant attentional and working memory load (VSEQ) and the other day a task of visuomotor learning with reaching movements (ROT). In both days, tasks were performed in blocks of 45 minutes in two 4-hour sessions, one in the morning and the other in the afternoon. After each block of either VSEQ or ROT, we assessed performance in a brief visual working memory test (*mem*), which shares the same neural substrates and characteristics as VSEQ and in a motor reaching test (*mot*), which taps into motor function akin to ROT. After the morning session, eight subjects slept for 90 minutes (NAP) and eight subjects rested quietly with eyes closed (REST). High density EEG was recorded throughout the experiment. In the last tests of the morning, performance in *mem* degraded more in the VSEQ session, while performance in *mot* was worse in the ROT sessions for both groups. In the afternoon sessions, *mem* performance specifically worsened in the REST group during the VSEQ session. Conversely, performance in *mot* worsened in the REST group during the ROT session. Analyses of the nap showed that slow wave activity, a measure of sleep intensity, was higher after the ROT task than after VSEQ with topographical differences between the two sessions. These results demonstrate that even, in absence of sleep deprivation, intense training during the day in normal subjects may lead to task-specific impairments, as performance decayed in the test that corresponded to the primary task performed. Most importantly, we found that a short nap but not a similar period of quiet rest protected against performance deterioration.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT Grant MAMG-I0007-2014-01-232334

Title: Insufficient sleep time is associated with low thyroid stimulating hormone levels in a sample of young mexican population

Authors: B. PÉREZ[1], R. SAÑUDO-TORRES[2], A. PAVÓN-ROSADO[1], S. ABURTO[1], R. AYALA-MORENO[2], *M. A. MELGAREJO[1];
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Abstract: Sleep is a necessary periodic process, and it also plays an important role in the normal function of the neuroendocrine system. Thyroid Stimulating Hormone (TSH) is a polypeptide hormone secreted by the thyrotrope cells of the anterior pituitary. Its plasma concentrations exhibit a circadian rhythm with a peak around the time of sleep onset. Chronic sleep deprivation is common in modern life; to be in this state implies that the nervous system is counteracting the sleep-wake cycle and body homeostasis. Some studies confirmed the consequences of this, but the relationship between thyroid function and sleep deprivation it remains unclear. Therefore the aim of the present study was to investigate associations between total sleep time and TSH levels in a sample of young Mexican Population. For this purpose a total of 69 students of Veracruzana University (18-25 years) participated. The study was conducted in according to standards established by the declaration of Helsinki as well as their applied the informed consent. All subjects completed a questionnaire Pittsburght for quality and quantity of total sleep. Thyroid Stimulating Hormone, Thyroxine (T4) and free thyroxine (FT4) levels were measured using commercial kits (Diagnóstica Internacional). Data were classified and analyzed with sigmaPlot 11.0. The results suggest that subjects who slept less than 6h presented low TSH levels (µUl/ml) (2.265±0.204 SEM) in comparison with the subjects who sleep more than 7h (3.016±0.241SEM) $p < 0.05$. In contrast, we did not find statistical differences between sleep hours with T4 and FT4. Our results suggest that the reduction of total sleep time may contribute to decrease Thyroid Stimulating Hormone levels and this condition might promote deregulation of neuroendocrine parameters at early age.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: 2014R1A1A2057866

Title: Aberrant brain functional network integrity in adolescents with insomnia

Authors: ***M.-H. PARK**[1], S. PARK[2], B. PARK[3], B.-N. KIM[4];
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Abstract: Background: It has been reported that 20-30% of adolescents suffer from insomnia. Previous studies showed that sleep problems are closely related to cognitive function such as memory, attention and executive function and that is supported by previous structural and functional neuroimaging studies.
Purpose: To investigate the whole-brain functional interactions and network organization of these interactions in adolescents with insomnia and control subjects.
Methods: We acquired resting-state functional MRI data from 47 adolescents with insomnia and 15 controls. Data were processed using standard procedures, and group analyses were performed using ANCOVA (covariates, age and gender) and nonparametric permutation tests. The severity of insomnia was measured by the insomnia severity index (ISI).
Results: The insular resting-state FC differences between adolescents with insomnia and control groups are displayed in Figures 1.
Some areas of FC showed negative correlations with the ISI total scores (Figure 2).
Conclusions: In this study, we showed several aberrant brain FC characteristics in adolescents with insomnia. These findings suggest that brain dysfunction in adolescents with insomnia extends to spontaneous resting conditions, and cognitive and affective deficits in adolescents with insomnia may stem from the altered FC and brain network organization, which may contribute to other psychiatric consequences and daily functional deficit associated with the condition.

Figure 1. Significantly decreased and increased functional connectivity in adolescents with insomnia over control subjects.

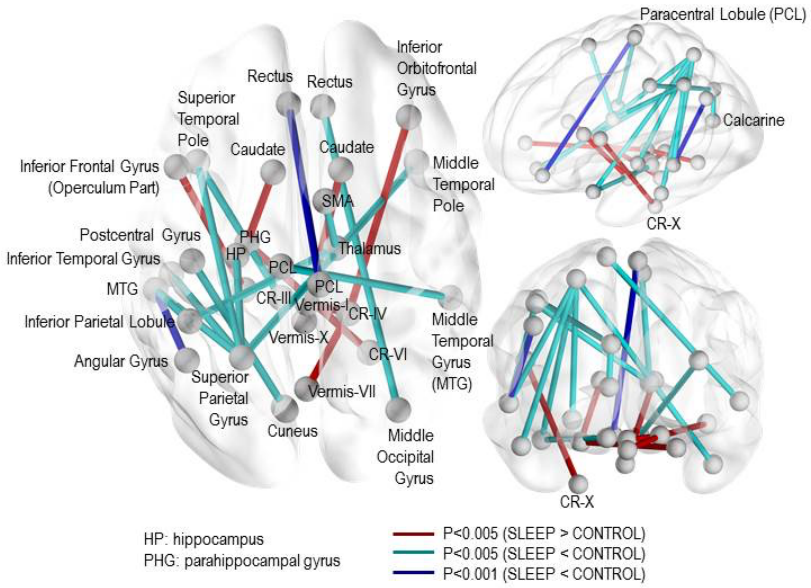
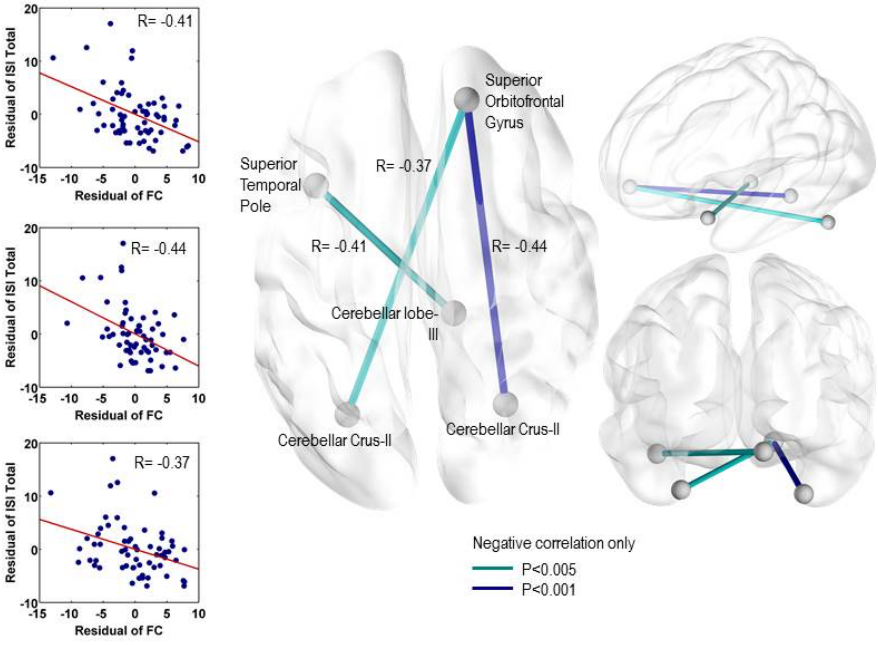


Figure 2. Correlation between functional connectivity and the ISI-total scores with age, sex covariates.



Disclosures: M. Park: None. **S. Park:** None. **B. Park:** None. **B. Kim:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: Start-Up Fund for New Recruits (1-ZE4M) to YSH.

Title: Investigation of sleep quality and executive functioning in Hong Kong adolescents

Authors: *J. Y. HO[1], Y. T. CHAN[1], A. K. C. SUEN[2], E. Y. Y. LAU[3], R. L. T. LEE[1], P. H. LEE[1], R. C. C. CHANG[4];
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Abstract: Sleep deprivation is believed to affect cognition in healthy adults but the effect of poor sleep quality on healthy young adolescent is unclear. We aim to investigate the effect of poor sleep quality on the executive functions of adolescents. In this pilot study, 47 junior Hong Kong high school students were recruited. They wore motionlogger watches and filled in sleep diary for 7 consecutive days and then completed five neuropsychological tests and a questionnaire for (1) a sleep-wake habit by Chinese Version of the Pittsburgh Sleep Quality Index (CPSQI), (2) Sleep Quality Index (SQI), (3) Epworth Sleepiness Scale (ESS), and (4) Depression Anxiety Stress Scale (DASS). Our results showed that over half of the students had poor sleep quality (58.8%, CPSQI less than 5); however, the mean of CPSQI is still marginally at score of 4.6. Less than half of the above students (45.8%) had a comparatively higher chance of dozing due to sleepiness by ESS results. DASS score demonstrated that students experienced mild-to-moderate depression and anxiety level (mean = 13.64). Adolescents with poor emotions and higher ESS score tended to have lower interference score in Stroop. Higher score in DASS, ESS and SQI were directly associated with the sleepiness in psychomotor vigilance test. Whilst DASS does not solely correlate to sleepiness in Psychomotor vigilance test (PVT) and The Stroop test, it also has a direct relationship with the number of too fast attempts in PVT. PSQI score is also positively-correlation with the number of moves and total time consumed in Tower of London test. From the results, sleep quality of the adolescents was just marginally normal. Their emotion was alarming and most of them exert depressed and anxious condition. We will further investigate how sleep quality affects executive functions.

Disclosures: J.Y. Ho: None. **Y.T. Chan:** None. **A.K.C. Suen:** None. **E.Y.Y. Lau:** None. **R.L.T. Lee:** None. **P.H. Lee:** None. **R.C.C. Chang:** None.

Poster

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A TDLC trainee award INCAW65 to Sue Peters

Title: Measures of cognition and sleep in infants, using dEEG: frontal sleep spindle spectral frequency is negatively correlated with cognition in 3.5-month-old infants

Authors: *S. PETERS, A. A. BENASICH;
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Abstract: The first year of life encompasses several major transitions with respect to both sleep and cognition, however, very little is known about how these two measures correlate over infancy. Infancy is a period of rapid change in the spectral components recorded from scalp EEG during sleep. These changes include the appearance of slow wave activity (SWA); the onset and peak of sleep spindle microstructure activity; and a transition from neonate sleep patterns to more adult-like sleep macrostructure (~ 3-months). Sleep spindles have long been considered a measure of maturation in infants; more recently SWA has been a focus of developmental sleep researchers, as a predictor of both cortical maturation and skill development. However, there is a pressing need for better understanding of the changing features of sleep spindles at these early ages, including their topography, frequency, and hemispheric synchronicity, particularly as characterized by dense-array EEG. Current literature is based on use of a minimal electrode array, and to date, no studies have measured concurrent sleep and behavior in a longitudinal sample. The present study aims to measure and characterize daytime sleep, concurrent with standardized behavioral measures of cognition, language, motor, and social-emotional development, in typically developing infants. Our design includes both cross-sectional and longitudinal groups, and here we describe results from a subset of 13 (6 female) longitudinal subjects at two ages: 3.5 and 6.5 months. Data is collected using 124-channel Hydrocel EEG nets (EGI, Inc.) during a 30 to 45 minute nap. Sleep macrostructure (REM and NREM) is scored, based on 17 anatomically mapped EEG channels and manually scored sleep behavior. Segments of stage 2 and 3 NREM sleep is extracted for analyses. Artifact-laden segments and channels are

removed, interpolated, and the data is referenced to a whole-head average. After FFT analyses, absolute power values are reported for standard frequency ranges, as spectrograms. Visually, clear maturational changes are seen in topography across the power spectrum, with some trait-like patterns appearing in the sigma (12-15Hz) band. We focus here on a cluster of frontal channels in the sigma range, which include the typical spindle frequencies, and match the peak activity from our sample. Within subjects, a significant maturational increase is seen in both peak frequency and power. Frontal spindle peak frequency is negatively correlated with cognitive performance at 3.5 months. This pilot study is ongoing, and is the first to characterize infant sleep spindle topography concurrent with behavioral measures in typically developing infants.

Disclosures: S. Peters: None. **A.A. Benasich:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

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R01 MH 107396

Leon Levy Foundation

Title: No entrainment of endogenous brain rhythms to 1Hz sinusoidal tACS in human intracranial EEG

Authors: B. LAFON[1], *L. C. PARRA[2], D. FRIEDMAN[3,4], S. HENIN[3,4], L. MELLONI[3,4], G. BUZSAKI[3,5], O. DEVINSKY[3,4], A. LIU[3,4];
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Abstract: Transcranial alternating current stimulation (tACS) is emerging as a non-invasive technique with applications in clinical therapy and cognitive research. Non-REM (NREM) sleep, and in particular slow-wave sleep (SWS), may support the consolidation of hippocampal dependent memories via the coordinated activity of slow oscillations (~1Hz) and spindles (~10-15 Hz). Previous studies argue that tACS applied during sleep may enhance the coordination between these rhythms to improve declarative memory recall. We sought to understand how applied transcranial electrical fields, delivered in an alternating or oscillatory manner (tACS)

would affect endogenous rhythms during NREM sleep, as measured in 4 patients undergoing invasive monitoring for epilepsy surgery. We expected that 1Hz sinusoidal tACS would entrain endogenous slow oscillations (0.75 Hz - 1 Hz) during sleep and modulate endogenous spindle activity power accordingly. We applied 1 Hz tACS at intensities ranging from 0.5 mA to 2.0 mA, using a frontal-occipital montage, in 4 patients undergoing invasive monitoring, during NREM sleep. Based on measurements and modeling, we estimated that the electrical fields at the cortical surface reach approximately 0.5 V/m, with maximal stimulation intensities of 2.0 mA. The interaction between spindle activity and slow oscillations was analyzed during endogenous sleep (across two different nights) and during 1Hz tACS. The cross frequency coupling between the slow and spindle rhythms was tested using a mean vector strength and spindle detection algorithm. During NREM sleep, spindle activity in 259 electrodes out of the 477 electrodes tested (among all subjects) was significantly modulated by endogenous slow oscillations, after correcting for multiple comparisons. Seventy five percent (75%) of the electrodes were consistently entrained from one night of sleep to the next. However, during applied TACS, spindle power was significantly modulated in only 3 out of the 477 electrodes tested. We conclude that 1 Hz TACS applied during sleep at commonly used stimulation intensities does not entrain spindle oscillations during NREM sleep. This negative finding may result from the weak electrical fields which reach the cortical surface.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT Grant MAMG- I0007-2014-01-232334

Title: Prevalence of sleep disorders in a mexican children population

Authors: ***S. ABURTO**, E. VENTURA-ARIZMENDI, B. PÉREZ, E. AGUILAR, R. MENDOZA-AMARO, A. NAVARRETE-MUNGUÍA, M. MELGAREJO;
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Abstract: An adequate good quality sleep is important for optimal health and functioning throughout life. In adults, reduced sleep duration and sleep disturbance have been demonstrated to be associated with cognitive deficits and mood disturbance. There is evidence that these same

effects occur in children but sleep duration and the potential consequence of sleep disturbance in children have received little attention in sleep studies. Several studies around the world have demonstrated a high prevalence of sleep disorders in children, being between 25 and 40 percent, sleep disorders varies with age and ethnicity, however the consequences seem to be universal, sleep disorders found associated with negative effects like poor school performance and changes in their social relationship, expressing irritability, mood swings and increased vulnerability to the adoption of addictive behaviors. Therefore, in practical terms, sleep disorders are associated with poor health and many psychosomatic symptoms. In Mexico, do not exist studies about the quality and sleep disorders in a child population; therefore the aim of the present study was to investigate the prevalence of sleep disorders in a sample of Mexican children population. For this purpose a total of 602 elementary school students (5-13 years) participated. The study was conducted according to the standards established by the declaration of Helsinki as well as their applied the informed consent. All subjects completed a questionnaire BEARS sleep screening algorithm, SDSC the Sleep Disturbance Scale for Children and PSQ Pediatric Sleep Questionnaire. 602 children participated in the study, 306 boys (50.8 percent, mean age = 8.36 ± 0.123) and 296 girls (49.3 percent, mean age = 8.38 ± 0.122). There were no differences in age by gender distribution ($p = 0.93$). The sleep disorders in relation to gender we observed that the prevalence is higher in males (35.94 percent) than females (29.39 percent). The results show that of the total population 32.7 percent have a sleep disorder, 44.6 percent of these have Sleep-Wake Transition Disorders, 40.6 percent Sleep Breathing Disorders and 26.3 Disorders of Excessive Somnolence. The subjects may be included in one or more groups because they can present more than one sleep disorder. The results indicate that the prevalence of sleep disorders in Mexican children correspond to statistics worldwide. However were highly prevalent the disorders in the transition of sleep-wakefulness. This high prevalence may conduct to learning and memory problems, even diseases such as obesity, diabetes mellitus and metabolic syndrome in an adult age.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.14/HHH7

Topic: F.08. Biological Rhythms and Sleep

Support: R01 HL109706

TR001082

Title: Spectral analysis of EEG activity during weekend recovery sleep

Authors: ***S. J. MORTON**[1,2], C. M. DEPNER[2], E. L. MELANSON[3,4], J. R. GUZZETTI[2], K. P. WRIGHT, Jr[2,4];
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Abstract: Many individuals obtain insufficient sleep at night during the work week and extend their sleep duration on the weekends in an attempt to recover. Sleep EEG changes in response to short sleep durations and total sleep deprivation (TSD). The impact of *ad libitum* weekend recovery sleep on sleep architecture has yet to be investigated. Thus, the aim of the current analysis was to investigate the effects of weekend recovery sleep on sleep architecture after a simulated work week of short sleep. Sleep was assessed using a combination of standard sleep staging and quantitative EEG power spectral analyses. 36 participants (18 females) aged 25.5±4.7y (mean±SD) were randomized into three study conditions, each lasting 13 days: control (9h sleep/night), sleep restriction (5h sleep/night), or weekend recovery (5 days of 5h sleep/night [simulated work-week], 2 days of *ad libitum* weekend recovery sleep with an enforced minimum of 10h time in bed [simulated weekend], followed by another 3 days of 5h sleep/night). Each condition began with 3 baseline nights (9h sleep/night). There was a significant interaction between night and hour of the sleep opportunity such that both delta and theta activity were significantly higher in the sleep restriction and weekend recovery conditions compared to baseline ($p < 0.05$). No significant changes in EEG activity were observed across days for the control condition. For all conditions, spectral analyses showed delta power was highest in the first three hours of the sleep opportunity and decreased thereafter. Overall, changes in delta and theta power during sleep restriction and weekend recovery sleep were consistent with higher homeostatic sleep pressure and recovery.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.15/HHH8

Topic: F.08. Biological Rhythms and Sleep

Title: Interaction of menstrual cycle and heart rate variability on sleep dependent memory consolidation

Authors: *N. SATTARI, L. WHITEHURST, M. NAJI, E. MCDEVITT, S. MEDNICK; UCR, Riverside, CA

Abstract: Sleep benefits the transformation of new information into long-term memories. Recent studies have indicated that menstrual phase may influence this process (Genzel et al., 2012). Also, studies have shown that menstrual phase may impact autonomic regulation during sleep (de Zambotti et al., 2013), and autonomic activity may also play a role in sleep-dependent memory consolidation (Whitehurst et al., 2016). Here, we investigated the interaction of menstrual cycle, autonomic activity measured by heart rate variability (HRV) on sleep-dependent memory consolidation. We used a declarative face-name association task (FNA) to compare memory performance in women during two phases of their menstrual cycle: 1) high hormones, WH (days 8 to 21) and 2) low hormones, WL (days 1 to 7 and 22 to 28), and men. 18 healthy females recorded their menstrual cycle daily for four weeks. In a within-subjects design, women completed a verbal recognition memory test before and after a 90-minute polysomnographically (PSG)-recorded nap on two separate occasions: high hormone and low hormone. We used paired t-tests to compare women in each phase on sleep and HRV variables as well as memory performance (d-prime). We also, used independent-samples t-tests comparing women in each phase to men (n=18). Bivariate correlations were used for sleep and HRV variables and performance. There was no difference in pre-nap performance (p=.8), but WH performed better than WL after the nap (p=.03). Post-nap d-prime was similar in WL and men (p=.6), whereas it was enhanced in WH compared to men (p=.02). There was no significant difference in sleep stages between WL and WH (all p>.05) but men had less REM sleep compared to WH (p=.03). HRV analysis revealed that men had higher total power (p=.07) in Stage 2 sleep along with more LF-HRV compared to WH (p=.05) reflecting increased sympathetic nervous system activity. HRV did not differ between women in both phases. In WH improvement in performance (pm-am) was correlated with both LF-HRV (r= .7, p=.008) and HF-HRV (r=.6, p=.02) in stage 2 as well as LF-HRV (r= .7, p=.03) and HF-HRV (r=.6, p=.04) in stage 3. We found that menstrual cycle and cardiac autonomic activity may influence the magnitude of sleep-dependent consolidation, whereby WH show less forgetting with a nap compared with WL while this improvement has a positive relationship with their sympathetovagal activity during Non-REM sleep. Compared with WH, men performed significantly worse on this paired associates memory

task, while showing increase sympathetic arousal during their nap. Our findings suggest that both sex hormones and HRV should be considered while looking at sleep-dependent memory consolidation.

Disclosures: **N. Sattari:** None. **L. Whitehurst:** None. **M. Naji:** None. **E. McDevitt:** None. **S. Mednick:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Program#/Poster#: 450.16/HHH9

Topic: F.08. Biological Rhythms and Sleep

Support: SFN

Title: Neural correlates of sleep-dependent consolidation of visual perceptual learning: an ERP study

Authors: ***M. AHMADI**[1], E. A. MCDEVITT[1], M. A. SILVER[2], S. C. MEDNICK[1];
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Abstract: Previous studies showed that both daytime naps and nighttime sleep facilitate consolidation of perceptual learning (PL) of a visual texture discrimination task (TDT) in humans. More specifically, this type of PL is retinotopically-specific and requires sleep to be consolidated (Stickgold et al., 2000; Mednick et al., 2003). Neuroimaging studies of TDT learning have shown changes in primary visual cortex (Walker et al., 2005; Pourtois et al., 2008) and in higher-level visual cortex, as well as changes in neural networks responsible for higher cognitive processes such as attention (Wang et al., 2016). In this study, we investigated how sleep facilitates PL by assessing neural changes associated with PL during encoding and retrieval of TDT. Subjects trained on TDT and, after two nights of sleep, they were tested on the same task. High-density electroencephalography (hdEEG) was recorded during encoding, retrieval, and sleep. We studied the ERP components C1 (early sensory processing), P1 and N1 (later sensory processing, modulated by top-down spatial attention) and P3 (cognitive processing). Our preliminary results show a significant decrease in C1 amplitude at retrieval, relative to encoding, in the parieto-occipital region (electrode POz). Although there were no significant changes in P1 amplitude with PL, N1 was significantly lower during retrieval at occipital and parietal sites. The difference in N1 amplitude between encoding and retrieval was positively correlated with behavioral threshold at right parietal cortex. We further analyzed P3 and found a significant increase in P3 amplitude associated with training at all parietal and occipital electrode sites.

Additionally, performance improvement was correlated with right lateralized ERP potentials when target was presented at right visual field. We conclude that sleep-dependent benefits of PL may be more related to changes in top-down attentional control and cognitive processes than to plasticity in early visual cortical areas.

Disclosures: M. Ahmadi: None. **E.A. McDevitt:** None. **M.A. Silver:** None. **S.C. Mednick:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01AG046646

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Title: Coupling of heart inter-beat intervals and slow oscillations during sleep

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Abstract: Introduction: The discovery of complex heart-brain neural interaction has prompted examination of the relationship between heart rate variability (HRV), a non-invasive measure of cardiac autonomic activity, and electroencephalography (EEG) during wake and sleep. In this study, we examined temporal coupling between EEG and heart inter-beat intervals (IBI, time-series of RR intervals, where R is one of component of ECG) during a daytime nap. **Methods**: 38 healthy subjects took a polysomnographically-recorded nap at 1:30-3:30PM. Slow oscillation (SO, <1 Hz) events during wake, Stage 2, slow wave sleep (SWS), and rapid eye movement (REM) sleep were identified based on the thresholding method. We then examined separately EEG/IBI activity time locked to the SO peaks. We aligned RR time-series (temporally smoothed by averaging in 0.5-sec bins) to half negative peak of the SO events in 10-second windows. We then compared changes in the averaged RR signal relative to the SO event with paired t-tests. Next, we aligned SO power to trough events in RR time-series. The correlation coefficient between average RR troughs and average SO power was calculated at the lag of the cross-correlation peak. **Results**: In Stage 2 sleep, we observed a 6.4% decrease ($p < .001$) in RR interval (or an increase in Heart Rate) beginning ~1 second *before* SO negative peak, reaching its minima ~2.5 sec after the negative peak. It was followed by a 3.8% increase ($p < .001$) in RR

interval until it reached a maxima ~5 sec after negative peak of SO. In stage 3, the relative timing of SO and RR was shifted and the amplitude of RR changes was smaller. Thus, RR interval started to decrease around time of SO negative peak until it reached it minima ~1.5 sec later (1.5% decrease, $p < .001$); it then reached its maxima ~6.5 sec after SO negative peak (1.0 % increase). In Stage 2 sleep, we discovered a correlation (0.86, $p < .001$) between power of SO and RR interval with peak delay of 0.66 sec; RR interval change was leading slow oscillation. Similarly, in Stage 3 correlation was 0.76 ($p < .001$) with 0.55 sec lag between SO power and RR interval change events. **Conclusion**: Our results demonstrate strong coupling between SO power and RR time-series during Stage 2 and SWS. Prior studies have reported RR changes follow SO events, however our analysis in contrast revealed that SO power follows changes in RR intervals after a delay. Further causality analysis is needed to understand the interactions between autonomic nervous system and central nervous system during sleep and wake.

Disclosures: **M. Naji:** None. **G.P. Krishnan:** None. **M. Bazhenov:** None. **S.C. Mednick:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: ONR Grant N00014-14-1-0513

Title: The impact of psychostimulants and hypnotics on cognitive performance in neurotypical adults

Authors: *L. N. WHITEHURST[1], L. BATELLI[2], S. AGOSTA[2], S. MEDNICK[1]; [1]Psychology, Univ. of California, Riverside, Riverside, CA; [2]Ctr. for Neurosci. and Cognitive Systems, Italian Inst. of Technol., Rovereto, Italy

Abstract: Introduction: Off-label use of psychostimulants (e.g. Adderall and Ritalin) for cognitive enhancement is becoming more prevalent in the US, particularly on college campuses. However, research examining the efficacy of these drugs as "smart pills" is mixed. Further, psychomstimulants decrease sleep, which has been shown to be important for cognition. As a result hypnotics are often used in conjunction with stimulants to promote sleep. To date, no studies have examined the combined effect of stimulants and hypnotics on sleep and cognition in healthy neurotypicals. We examined the effects of a single dose of dextroamphetamine (DAMP vs. placebo (PBO)) during the day and zolpidem (ZOL vs PBO) during the night on verbal

memory and sustained attention, in a double-blind, placebo controlled, cross-over design.
Methods: 20 (11F) healthy subjects were tested in four conditions (DAMP/PBO, DAMP/ZOL, PBO/ZOL, PBO/PBO), one week apart. On each day, baseline performance was obtained for the word pair associates task (WPA1, subjects encoded 60 word pairs, followed by an immediate test) and a multiple object tracking task (MOT1, visual tracking of multiple moving targets among distractors). Drug (20 mg of DAMP or PBO) was administered at 9:00AM and MOT was retested 75 minutes post-drug (MOT2). At 9:00PM, recall for 20 word pairs (WPA2) and MOT (MOT3) were tested. Subjects were either administered 10mg of ZOL or PBO, and slept overnight in the lab while they were EEG-monitored. The next morning, subjects completed a final session of WPA3 and MOT4 tasks. We calculated change in performance across sessions with a difference score for both tasks.
Results: We found no effect of DAMP on WPA over 12 hours of wake compared with PBO ($p = .26$). After sleep, the PBO/ZOL group performed better than all other groups ($p = .029$), with no differences in other conditions. Additionally, DAMP/PBO showed the worst performance after a 12-hour overnight sleep period ($p = .09$). For MOT, the DAMP condition showed a 10% increase in performance at MOT2 compared to PBO ($p = .037$) and this performance boost persisted over 12 hours (MOT3, $p = .08$). However all benefits of DAMP for MOT disintegrated after sleep ($p = .33$).
Conclusion: Here, we show that healthy young adults show task specific enhancement with psychostimulants, with increased attentional processing but no benefit on declarative memory. Interestingly, these drug benefits did not persist following a night of sleep. These data suggest there may be important interactions between stimulant drugs and sleep-related consolidation that should be considered.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: NSF 1439210

Title: Modulating acetylcholine during sleep consolidation of episodic memory and perceptual learning

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Abstract: The neurotransmitter acetylcholine (ACh) regulates consolidation of both episodic memory and perceptual learning (PL), but previous studies suggest a different role for ACh for these two types of memory (Gais et al., 2004; Rokem & Silver, 2010). Specifically, low levels of ACh, as in non-rapid eye movement (non-REM) sleep, may be optimal for episodic memory consolidation (Hasselmo & McGaughy, 2004), whereas increased cholinergic transmission, as in REM sleep, is hypothesized to set the appropriate neural dynamics for consolidation of PL (Mednick et al., 2003). We assessed the effects of cholinergic enhancement on sleep consolidation of episodic memory and visual PL by administering the cholinesterase inhibitor rivastigmine, which increases synaptic levels of ACh, during a period of overnight sleep. Twenty young, healthy subjects participated in a randomized, double-blind, placebo-controlled crossover study. On the morning of Day 1, subjects were trained on a declarative (word paired-associates) task and non-declarative PL (texture discrimination) task. Rivastigmine (1.5 mg) or placebo was then administered before electroencephalogram (EEG)-recorded sleep on Night 1. On the morning of Day 3, after a second night of sleep with no additional drug, subjects were tested on both tasks. For word paired-associates, immediate recognition test performance was greater than chance ($p<.001$) in both drug conditions, indicating that subjects learned the word pairs. Across days, there was a significant amount of forgetting ($p<.001$), but recognition performance remained above chance on Day 3 ($p<.001$). However, there was no difference in the amount of forgetting between drug conditions ($p=.2$). For texture discrimination, thresholds improved between Days 1 and 3 ($p=.02$), but there was no effect of cholinergic enhancement, with rivastigmine and placebo conditions showing equivalent magnitude of PL ($p=.9$). In summary and contrary to our hypotheses, there was no effect of cholinergic enhancement for sleep consolidation of either episodic memory or PL. Further analysis of sleep EEG recordings will allow assessment of the impact of rivastigmine on sleep features. Additionally, we will verify whether our results replicate with a 24hr retention interval that includes only one night of sleep following drug administration. It is possible that a single low dose of a cholinesterase inhibitor is not effective for boosting ACh during sleep. Another possibility is that our behavioral measures were not sensitive enough to detect an effect of cholinergic enhancement on learning. Further research is needed to elucidate the complex relationships among sleep, memory and ACh.

Disclosures: E.A. McDevitt: None. **M. Ahmadi:** None. **M.A. Silver:** None. **S.C. Mednick:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Program#/Poster#: 450.20/HHH13

Topic: F.08. Biological Rhythms and Sleep

Support: Judith Jane Mason Foundation

Title: Medial prefrontal white matter changes associate with sleep quality in patients with Chronic Fatigue Syndrome

Authors: *Z. SHAN[1], R. KWIATEK[2], R. BURNET[3], P. DEL FANTE[4], D. R. STAINES[1], S. M. MARSHALL-GRADISNIK[1], L. R. BARNDEN[1];
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Abstract: Introduction: Chronic fatigue syndrome (CFS), sometimes referred to as myalgic encephalomyelitis (ME), is a debilitating illness with a variety of presenting features. Unrefreshing sleep is one of commonly observed features in CFS patients. However, the brain structural substrate of unrefreshing sleep in CFS remains elusive. This study explored the relationship between brain structure and sleep quality in patients with CFS.**Methods:** 38 patients with CFS (34.14 ± 9.85 years old) and 13 normal controls (NCs) (34.8± 8.73 years old) were recruited. All subjects completed the Hospital Anxiety and Depression Scale (HADS) and the Pittsburgh sleep quality index (PSQI) questionnaire. Brain magnetic resonance imaging (MRI) yielded global and regional grey matter (GM) and white matter (WM) volume images from voxel based morphometry, magnetization transfer contrast (MTC) T1 weighted intensities, T1 weighted (T1w) and T2 weighted (T2w) spin-echo signal intensities. We tested for inter-group differences and correlations between each pair of these measures. Voxel based regressions of regional brain MRI measures were performed with the PSQI scale adjusted for age, anxiety and depression, and the appropriate global measure. **Results:** There was no significant difference between CFS patients and NCs in total GM volumes ($P = 0.41$), total WM volumes ($P = 0.68$), or age ($P = 0.82$). CFS patients had significantly higher PSQI (lower sleep quality) ($P < 0.001$), anxiety ($P = 0.005$) and depression ($P < 0.001$) than NCs. Total GM volumes were significantly correlated with total WM volumes (0.65, $P < 0.001$) and age (-0.48, $P < 0.001$). The PSQI scales were significantly correlated with depression (0.48, $P < 0.001$) and anxiety (0.45, $P< 0.001$). The depression scales were significantly correlated with anxiety scales (0.63, $P < 0.001$). Regionally, we found a significant decrease in WM volumes (family wise error adjusted cluster level P value, $P_{FWE} < 0.05$) in the left inferior fronto-occipital fasciculus (IFOF) in CFS patients. In CFS patients, negative correlations were observed between PSQI and MTC intensities ($P_{FWE} < 0.05$)

and between PSQI and T1w intensities ($P_{FWE} < 0.05$) in the medial frontal cortex. The same correlations were observed when CFS patients and NCs were pooled. In the same medial frontal cortex location, both MTC and T1w intensities were lower in CFS patients compared with NCs (uncorrected $P < 0.001$).**Conclusion:** These findings confirmed WM deficits at the IFOF in CFS patients reported in our previous longitudinal study. This study is the first to report that sleep disruption in CFS is mediated by brain structural differences.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.21/HHH14

Topic: F.08. Biological Rhythms and Sleep

Title: Sleep apnea symptoms and depression in young adults

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Abstract: The purpose of this study was to examine both sleep apnea symptoms and depressive symptoms in a sample of 93 students enrolled at Southern Adventist University. Specifically, we examined the linear relationship between sleep quality and depressive symptoms as expressed by the students and compared their scores on both depression and sleep quality to their membership in certain groups: age, gender, ethnicity and academic major.1. There will be gender differences in depressive symptoms. Women will report more depressive symptoms than men. 2. What is the linear relationship between sleep and depressive symptoms? 3. What is the linear relationships among age, depressive symptoms, and sleep? 4. Are there gender, ethnic, and academic major differences in sleep and depressive symptoms? The sample of convenience consisted of 93 student participants (42 male), median age 21 (range 18 to 53), representing mainly Humanities academic discipline, and 3 major ethnic categories: White, Black/African American, Hispanic/Latin/a, Other Race designations. Results showed that1. There will be gender differences in depressive symptoms. Women will report more depressive symptoms than men. 2. What is the linear relationship between sleep and depressive symptoms? Yes, positive and statistically significant ($r(93) = .55$, $p = .000$) explain r-squared.3. What is the linear relationships among age, depressive symptoms, and sleep?Older students have more sleep quality problems; age not related to depression,4. Are there gender, ethnic, and academic major differences in sleep

and depressive symptoms? No (ns), although women report more sleep quality problems and show more depressive symptoms and Blacks had the poorest sleep quality scores and Hispanics the lowest depressive symptoms. Humanities had poorer sleep quality than other majors but lower depressive symptoms.

Disclosures: R. Williamsmorris: None. **F. Barrientos:** None. **T. Reynoso:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Program#/Poster#: 450.22/HHH15

Topic: F.08. Biological Rhythms and Sleep

Support: Herbert T. Abelson Chair in Pediatrics

Title: Altered regional cortical thickness in children with obstructive sleep apnea syndrome

Authors: *R. A. MA[1], L. KHEIRANDISH-GOZAL[3], M. F. PHILBY[4], R. KUMAR[2,5], D. GOZAL[3], P. M. MACEY[1,5];
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Abstract: Introduction: Pediatric obstructive sleep apnea syndrome (OSAS) is highly prevalent condition, and is associated with increased risk of impaired cognitive performance. Adults with OSAS manifest brain injury linked with their clinical symptoms, and animal studies mimicking OSAS, lead to regional neuronal losses, suggesting that pediatric OSAS patients may also show brain alterations. In an earlier study, we found that regional gray matter volume was reduced in selected cortical and brainstem regions in OSAS children. In the present study, we aimed to assess regional cortical thickness changes in pediatric OSA patients over controls.
Methods: We evaluated 16 pediatric OSAS participants (8 male, mean age (±SD) = 8.1±2.2 years), with polysomnographic evidence of OSA (AHI=11.1±5.9 events/hr), and 141 control subjects (64 male, 8.2±2.0 years), 132 of whom were from the NIH Pediatric MRI database, and others were matched in age, gender, ethnicity, and BMI. High resolution T1-weighted images were assessed for regional cortical thickness alterations using FreeSurfer software. Comparisons between OSAS and control groups were performed vertex-by-vertex using ANCOVA, with age and gender as covariates ($P<0.05$, false discovery rate correction for multiple comparisons).
Results: Regional cortical thinning in OSAS group was present in the superior and medial

frontal, prefrontal, and parietal cortices, and medial occipital cortex, consistent with the previously identified locations of gray matter volume reduction. Additionally, multiple isolated regions of increased cortical thickness in OSAS patients appeared in the bilateral precentral gyrus and left central gyrus, and small regions in the insular cortices. The posterior cingulate and sub-genu of the anterior cingulate cortices, extending into medial prefrontal areas also showed increased thickness in OSAS, as did the medial temporal lobes.
Conclusions: Pediatric OSAS is associated with regions of thinner cortex, consistent with chronic changes including neuronal and cellular loss, and isolated areas of increased cortical thickness, suggesting acute tissue changes including inflammatory or glial responses to ongoing hypoxic exposures or fragmented sleep. Since the duration of the disease in OSAS patients is unknown, the pathological processes leading to cortical thinning may have been operational for several years. The short and long-term consequences of these cortical changes and their reversibility with therapy remain to be delineated.
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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: National Institute of Nursing Research NR-013693

Title: Nucleus accumbens regional volume changes in newly-diagnosed obstructive sleep apnea patients vary by sex

Authors: *J. PRASAD[1], J. A. OGREN[2], R. KUMAR[3,4], R. AVSOLA[5], F. L. YAN-GO[6], M. A. WOO[7], M. THOMAS[8], R. M. HARPER[9,4], P. M. MACEY[10,4];
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Abstract: Introduction: Obstructive sleep apnea (OSA) patients experience emotional dysregulation, such as anhedonia accompanying depressive symptoms, which is present in half of these patients. The shell of the nucleus accumbens (NAc) helps mediate pleasurable feelings,

as well as aversion and maternal behavior. Several adjacent brain structures are damaged in OSA, but it is unclear whether the NAc, or its subregions are affected. We used surface-based analysis to assess subregional NAc volume differences, and evaluated those differences by sex, since depression aspects differ between males and females in OSA. **Methods:** We collected high-resolution T1-weighted images from 66 newly-diagnosed, untreated OSA (mean age±SD: 46.3±8.8yrs; mean AHI±SD:34.1±21.5 events/hour; 30 male) and 59 healthy control (46.8±9.0 yrs; 38 male) subjects. We combined T1-weighted images (1.0 mm^3 resolution) of healthy controls from two large MRI datasets (IXI and OASIS), for a total of 979 controls (46.5±9.9 years; 426 male). We used "FSL FIRST" software to segment the NAc and assess regional surface-based structure. NAc volumes were scaled for total brain size, based on registration to a common space (6 parameter affine registration), and included age and total intracranial volume (TIV) as covariates (ANCOVA; $P<0.05$, permutation testing correction for multiple comparisons). **Results:** Left and right NAc showed regional volume increases in male, but not female OSA over controls, accounting for head size, TIV, and age ($P<0.05$, corrected for multiple comparisons). The left NAc showed a small surface region (4 mm^2) of increase in the superior lateral mid-NAc, likely reflecting core changes, with a maximum effect size in OSA of 0.20 mm. The right NAc showed extensive surface regions (61 mm^2) of volume increase across the anterior-inferior NAc, likely reflecting shell changes in the lateral, anterior and medial areas, and core in the mid-anterior, inferior portion, with some increases extending superiorly in the mid-to-posterior aspect. The maximum effect size was 0.38 mm in the inferior aspect.**Conclusions:** The NAc in OSA shows increased regional volumes in male, but not female OSA patients, with the right structure especially affected. Volume increases suggest inflammation and glial activation; absence of volume decreases suggests little long-term injury in OSA. The reasons for the sex differences are unclear. However, given the greater levels of anhedonia-related symptoms in female OSA patients, the findings suggest that volume change and relationship to loss of pleasurable feelings are not simple, and likely involve multiple other brain regions.

Disclosures: J. Prasad: None. **J.A. Ogren:** None. **R. Kumar:** None. **R. Avsola:** None. **F.L. Yan-Go:** None. **M.A. Woo:** None. **M. Thomas:** None. **R.M. Harper:** None. **P.M. Macey:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.24/HHH17

Topic: F.08. Biological Rhythms and Sleep

Support: National Institute of Nursing Research NR-013693

Title: Obstructive sleep apnea is accompanied by altered neurochemical levels in the midbrain and hypothalamus

Authors: *P. M. MACEY[1,2], M. K. SARMA[3], R. NAGARAJAN[3], J. A. OGREN[4], R. AYSOLA[5], R. M. HARPER[4,2], M. A. THOMAS[3];
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Abstract: Introduction: Obstructive sleep apnea (OSA) is associated with structural alterations in brain tissue and altered neural function in both limbic and cortical regions as well as brainstem areas and the cerebellum. Since the midbrain and adjacent hypothalamus contain many integrative nuclei, they are critically involved in physiological functions that are disrupted in OSA. To directly identify whether the functional state of these regions is altered in OSA, we assessed neurochemical levels within the midbrain/hypothalamic region. **Methods:** We used two-dimensional magnetic resonance spectroscopy (2D-MRS) to measure multiple neurochemicals in the midbrain/hypothalamus of OSA and control patients. We studied 14 OSA patients (mean age±SD:54.6±10.6years; AHI:35.0±19.4;SAO_2min:83±7%), and 26 healthy control participants (50.7±8.5years) using a recently-developed non-uniform undersampled (NUS) compressed sensing-based four dimensional echo-planar J-resolved spectroscopic imaging (4D-EP-JRESI), which enables evaluation of more metabolites than traditional 1D MRS/MRSI. We localized the midbrain using high-resolution T1-weighted anatomical scans. Acquired data were post-processed with a custom MATLAB-based program, and metabolite ratios with respect to creatine peak were calculated using a modified prior knowledge fitting (ProFit) algorithm. Independent samples t-tests were used to assess OSA-control differences. **Results:** In the right midbrain and hypothalamus, the OSA group showed decreased N-acetylaspartate (NAA; OSA: 1.24±0.43, Control: 1.47±0.41; p=0.03), a marker of neuronal viability. Increases in OSA over control subjects appear in levels of glutamate (Glu; OSA: 1.23±0.57, Control: 0.98±0.33; p=0.03), ascorbate (asc; OSA: 0.56±0.28, Control: 0.42±0.20; p=0.03), and myo-inisotol (mI; OSA: 0.96±0.48, Control: 0.72±0.35; p=0.03). No differences between groups appeared in γ-aminobutyric acid (GABA) or taurine (Tau). **Conclusion:** The midbrain and hypothalamus in OSA patients show decreased NAA, which likely indicates neuronal injury. Higher Glu levels may reflect excitotoxic processes, and higher mI is consistent with glial activation, as that chemical is prominant in such cells. Asc may be high in response to the oxidative stress from intermittent hypoxia in OSA. Additionally, Asc and Glu are both involved with glutamatergic processes, which are likely present in the midbrain in OSA patients.

Disclosures: P.M. Macey: None. **M.K. Sarma:** None. **R. Nagarajan:** None. **J.A. Ogren:** None. **R. Aysola:** None. **R.M. Harper:** None. **M.A. Thomas:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.25/HHH18

Topic: F.08. Biological Rhythms and Sleep

Title: Wake high-density EEG spatiospectral signatures of insomnia

Authors: *E. J. VAN SOMEREN[1], M. COLOMBO[2], Y. WEI[2], J. RAMAUTAR[2];
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Abstract: Objectives. Insomnia is characterised by fragmented sleep and hyperarousal. Whereas diagnostic criteria include daytime complaints, studies mostly focused on sleep EEG. We systematically evaluated wake high-density (HD) EEG signatures of insomnia during resting state and in response to external (auditory) and internal (heartbeat) stimuli.
Methods. Fifty-four people with insomnia were compared to 48 controls without sleep complaints, recruited from www.sleepregistry.nl. During the evening hours, HD-EEG was assessed using a 256-electrode system during an active auditory oddball task and during 5 minutes of eyes open and 5 minutes of eyes closed resting-state. Simultaneous electrocardiography allowed for heartbeat evoked potentials (HEP) analyses.
Results. During adaptation to frequent tones, the auditory N100 amplitude decreased in controls but not in people with insomnia. Heartbeat evoked potential analysis indicated that eye closure attenuated the frontal HEP amplitude in a late (372-492 ms) time window. In people with insomnia, eye closure attenuated this late part of the HEP amplitude significantly less. Resting-state spectral power analyses indicated that insomnia is characterised by low power in the upper alpha range (11-12.7 Hz) during eyes open over restricted bilateral frontal and left temporal regions. During eyes closed, people with insomnia show high power in the beta-gamma range (16.3 to 40 Hz) over extended prefrontal, central and parieto-occipital regions.
Conclusions. Increased high-frequency power in insomnia is not limited to sleep-EEG, suggesting a 24-hr deficiency to suppress cortical activation. This interpretation is also supported by the attenuated power in the upper alpha band, given the inhibitory role of alpha. Auditory ERP findings suggest that people with insomnia insufficiently adapt to frequent stimuli. The lack of adaptation is also supported by the enhanced late HEP amplitude.

Disclosures: E.J. Van Someren: None. **M. Colombo:** None. **Y. Wei:** None. **J. Ramautar:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 450.26/HHH19

Topic: F.08. Biological Rhythms and Sleep

Support: NHMRC (Australia)

ANZ Trustees

Title: "Somnivore", a user-friendly platform for automated sleep scoring of animal and human polysomnography data

Authors: *G. ALLOCCA[1], L. A. JOHNSTON[1], D. R. FREESTONE[2], A. L. GUNDLACH[1]; [1]Florey Dept. of Neurosci. and Mental Hlth., [2]Med., The Univ. of Melbourne, Parkville, Australia

Abstract: Objective: The low-throughput nature of manual scoring of sleep data is a major factor restricting the pace and potential of sleep research. Automated scoring approaches developed previously have failed to provide sufficient accuracy or 'usability' for sleep scientists lacking engineering or specialist computing expertise. Moreover, all previous approaches have only been validated on baseline data and no single approach has been validated for mouse, rat and human data analysis. Methods: We have developed a user-friendly platform for real-time automated scoring of polysomnography data, named Somnivore. Using a GUI-based approach in the Matlab™ platform we have deployed a support vector machine (SVM) to analyse features from polysomnography inputs (EEG, EMG, EOG, ECG, temperature, etc.) into the various sleep stages. The SVM is trained for each individual subject via a brief session of manual scoring. The system has been validated using mouse, rat and human data collected across a number of different treatments and genotypes and scored by members of collaborating laboratories from Australia, Europe and USA. Results: With minimal training time, overall scoring agreement rates were consistently above 90% in all species and treatment groups. F-scores in rats and mice were >0.9 for wake, >0.9 for NREM and >0.85 for REM. For human data, F-scores were >0.85 for wake, >0.35 for N1, >0.88 for N2, >0.85 for N3 and >0.91 for REM.Conclusions: Somnivore provides accurate, reliable, high-throughput scoring and analysis of polysomnography data in a range of experimental situations from normal and genetically modified physiology to drug pharmacology, in both animal and human subjects.

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Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01 MH099645

ONR N00014-13-1-0672

National Science Foundation Graduate Research Fellowship

Chateaubriand Fellowship

Title: Coordination of cortical and thalamic activity during non-REM human sleep

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Abstract: Every night the human brain produces thousands of slow oscillations and spindles during non-REM sleep. Previous studies indicate that slow oscillations generated in the cortex group spindles generated in the thalamus. Cortico-thalamic mechanisms underlying these sleep phenomena, however, remain poorly characterized in humans. Using simultaneous cortical and thalamic bipolar depth recordings in patients undergoing evaluation for epilepsy (2 women, 1 man), we demonstrate a cortico-thalamo-cortical loop whereby: 1) cortical slow oscillation downstates lead thalamic downstates; 2) thalamic downstates hyperpolarize thalamic cells, thus triggering spindles; and 3) thalamic spindles are focally projected back to cortex. In earlier models, thalamic H and T currents were assumed to be continuously available, permitting spontaneous spindling; our findings indicate, in contrast, that thalamic currents are not available until the cells are further hyperpolarized in a thalamic downstate. In earlier models, cortical spindle occurrence was supposed to be gated by cortical upstates. In contrast, we find that spindle occurrence is gated by the thalamic downstate, and thus, cortical spindles begin on the down-to-upstate transition when their contribution to memory consolidation can be maximized.

This archetypical cortico-thalamo-cortical sequence provides the global physiological context for memory replay and consolidation during non-REM sleep.

Disclosures: R.A. Mak-Mccully: None. **M. Rolland:** None. **A. Sargsyan:** None. **J. Trees:** None. **P. Chauvel:** None. **H. Bastuji:** None. **M. Rey:** None. **E. Halgren:** None.

Poster

450. Sleep Behavior in Humans and Non-Human Primates

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01 NS036449

ONR MURI N000141310672

HHMI

NIH 5T32EY20503-5

BIAL 220/12

Title: Repeating circular waves enable strengthening of large-scale neural assemblies during sleep spindles in human cortex

Authors: *L. E. MULLER[1], G. PIANTONI[2], D. KOLLER[1], S. S. CASH[2], E. HALGREN[3], T. J. SEJNOWSKI[1];
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Abstract: During sleep, the thalamus generates a characteristic pattern of transient, 11-15 Hz sleep spindle oscillations, which synchronize the cortex through large-scale thalamocortical loops. Spindles have been increasingly demonstrated to be critical for sleep-dependent consolidation of memory, but the specific neural mechanism for this process remains unclear, particularly in the presence of long axonal conduction delays. Evidence from human electroencephalography (EEG) and animal recordings suggests large-scale coherence occurs across the cortex during spindles in natural sleep, but their specific spatiotemporal structure is not well understood. In this work, we study electrocorticogram (ECoG) recordings of patients during stage 2 sleep and apply computational methods to characterize spatiotemporal dynamics.

We find that cortical spindles are spatiotemporally organized into circular wave-like patterns, traveling preferentially in the temporal-parietal-frontal (TPF) direction. These activity patterns extend over tens of milliseconds, placing the neural assemblies they synchronize on a timescale relevant to spike-time dependent synaptic plasticity. The waves travel with a peak speed between 3-5 m/s, within the range of conduction speeds of the short and long association fibers, allowing spikes emitted in distant cortical areas to align with the local spindle-associated population burst. Finally, we show these circular patterns repeat over hours of sleep with millisecond temporal precision, enabling reinforcement of large-scale neural assemblies through hundreds of reverberations. These results provide a novel mechanistic account for how global sleep oscillations and synaptic plasticity can strengthen networks distributed across the cortex to store coherent and integrated memories.
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Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.01/HHH22

Topic: G.01. Appetitive and Aversive Learning

Title: The influence of intra-dorsolateral striatum injection of an NMDA receptor agonist or antagonist on extinction of response learning in the plus-maze

Authors: *J. GOODMAN, R. RESSLER, M. G. PACKARD;
Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Extensive evidence indicates that acquisition of response learning is critically mediated by the dorsolateral striatum (DLS). Recent evidence from our laboratory indicates that, like initial acquisition, extinction of response learning may similarly depend on DLS function. However, the precise neurotransmitter systems involved remain unknown. NMDA receptor activity has been critically implicated in multiple kinds of learning and memory, including initial

acquisition of response learning and extinction of conditioned fear. Thus, the present study examined whether NMDA receptor activity in the DLS may also be implicated in extinction of response learning. Adult male Long-Evans rats were initially trained in a response learning version of the plus-maze, in which they were reinforced to make a consistent turning response (e.g. turn left) at the choice point to retrieve food reinforcement at the end of a separate goal arm (East or West). Following initial acquisition, animals were given two days of extinction training, which was conducted in a manner identical to initial acquisition except without the reinforcer present. In experiment 1, immediately after the first day of extinction training, animals received bilateral intra-DLS infusions of the NMDA receptor antagonist AP5 (2μg/kg) or saline. On the second day of extinction training, animals that had received post-training AP5 displayed more perseverative turning responses and lower running latencies, relative to animals that had received post-training saline. Experiment 2 was conducted in a similar manner, except animals received post-training intra-DLS administration of the NMDA receptor partial agonist D-cycloserine (DCS; 10μg or 20μg/kg dose) or saline. Animals given 20μg DCS, but not 10μg DCS, displayed higher latencies and fewer perseverative turning responses, relative to animals given post-training saline. The present findings indicate that NMDA receptor activity in the DLS governs bidirectional effects on extinction of response learning. Whereas blocking DLS NMDA receptors with AP5 impairs extinction of response learning, stimulating NMDA receptors with D-cycloserine enhances extinction of response learning. Considering that DLS-dependent habit memory may underlie some symptoms of human neuropsychiatric disorders (e.g. drug addiction), the present findings may be useful in developing pharmacological treatments that combat the habit-like behavioral features of these disorders.

Disclosures: **J. Goodman:** None. **R. Ressler:** None. **M.G. Packard:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.02/HHH23

Topic: G.01. Appetitive and Aversive Learning

Title: Effectiveness of extinction protocols depends on the memory system engaged during acquisition

Authors: ***R. RESSLER**, J. GOODMAN, M. PACKARD;
Psychology, Texas A&M, College Station, TX

Abstract: Previous research suggests that extinction of learned maze behavior in rodents may occur without the overt performance of the previously acquired response. Previous studies have

demonstrated that confining an animal to a previously rewarded goal location in the absence of reward, a protocol known as latent extinction is sufficient to produce the extinction of behavior. Despite this, it is unknown the latent extinction protocol is effective in extinguishing all types of memory acquired in a maze. In this study we examined the effectiveness of latent extinction in two separate plus-maze tasks, each of which was designed to engage anatomically distinct neural constructs. First, adult male Long-Evans rats were trained in a hippocampus-dependent place learning task. In this task animals are begun from different starting positions (North/South) and are trained to approach the same spatial location (East) in order to obtain a food reward. In another experiment, a separate group of rats was trained in a dorsolateral striatum-dependent response learning task in which rats were again started from different positions and were required to make a consistent egocentric body-turn response to obtain the food reward. After training, the animals received either latent or response extinction. For latent extinction animals were confined to the previous goal location in the absence of reward. For response extinction, animals were given the opportunity to execute the original running approach response toward the empty goal location. The results indicate that relative to no extinction, latent extinction was effective in extinguishing memory acquired in a place learning task but was ineffective in extinguishing memory acquired in a response learning task. In contrast, response extinction was effective in extinguishing memory acquired in both the place and response learning tasks. These results suggest that the extinction of maze behavior may occur without the explicit performance of the previously acquired response, as is the case with latent extinction, however the effectiveness of this protocol may be dependent on the type of memory being extinguished. The present findings suggest that behavioral treatments, specifically those modeled after response extinction protocols, may be particularly useful in treating human psychopathologies associated with striatum-dependent processes (e.g., drug addiction and relapse).

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Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.03/HHH24

Topic: G.01. Appetitive and Aversive Learning

Title: Selective effects of dorsal and ventral medial prefrontal cortex inactivation during instrumental reward seeking

Authors: *J. P. CABALLERO[1], D. E. MOORMAN[1,2];
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Abstract: Previous research has shown that, in rodent medial prefrontal cortex (mPFC), the dorsal mPFC (typically prelimbic) promotes the expression of conditioned fear and drug-seeking behavior, and the ventral mPFC (typically infralimbic) promotes inhibition of these behaviors. However, both ventral and dorsal mPFC are activated during reward seeking and extinction, and these established roles may not apply to all forms of motivated behavior. Additionally, this model does not take into account possible mPFC hemispheric differences which some groups have demonstrated previously. In order to better understand the roles of dorsal and ventral mPFC in motivated behaviors we performed bilateral and unilateral pharmacological inactivation of dorsal and ventral mPFC during reward seeking, fear conditioning, and extinction learning. Male Long-Evans rats were first trained to perform a fixed ratio 1 (FR1) sucrose seeking task where each nosepoke resulted in a tone cue and the delivery of 0.1 ml 15% sucrose. Responding was extinguished following acquisition of stable task performance. After extinction, rats received cue-induced reinstatement of sucrose seeking. During each phase of the task, rats received bilateral dorsal or ventral mPFC inactivation, or inactivation of only left or right dorsal or ventral mPFC. A subset of rats then underwent cued fear conditioning in which a new set of tone cues signaled footshock. Following conditioning, rats underwent extinction learning and recall. Rats received bilateral inactivations during extinction learning. During FR1 sucrose seeking we observed no effect of inactivation of either dorsal or ventral mPFC on numbers of nosepokes or well entries. Inactivation of left, right, and both hemispheres of dorsal mPFC decreased latency between nosepoke and reward collection ($p < 0.0001$ in all cases). Bilateral inactivation of ventral mPFC also decreased nosepoke-to-reward reaction time ($p<0.05$), as did left ($p<0.0001$) but not right ($p=0.0964$) hemisphere inactivation. Sucrose extinction, reinstatement and fear conditioning data are currently being analyzed. These preliminary results argue against a general hypothesis that dorsal or ventral mPFC selectively drive execution or inhibition of all motivated behaviors. Instead, they suggest that these areas may influence certain aspects of motivated behavior such as speed-accuracy tradeoff. Our results also demonstrate possible differential hemispheric contributions of mPFC subregions during reward seeking. Further analysis will confirm the extent of these subregion-by-hemisphere interactions in different aspects of motivated behavior.

Disclosures: J.P. Caballero: None. **D.E. Moorman:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.04/HHH25

Topic: G.01. Appetitive and Aversive Learning

Support: BFU2014-56692-R

Title: When and where learning is taking place? Multisynaptic changes in strength during different behaviors related to the acquisition of an operant conditioning task by behaving rats

Authors: *J. DELGADO-GARCIA;
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Abstract: Although it is widely assumed that brain circuits are modified by new experiences, it is still unanswered which types of change in synaptic efficacy takes place in cortical and subcortical circuits during the very moment of the acquisition process. Rats were trained to the acquisition of an operant conditioning task using a fixed ratio (1:1) schedule. Animals were trained in a modified Skinner box provided with light beams to detect animals' approaches to the lever and to the feeder. Behaviors related (as approaching the lever, pressing the lever, eating) and unrelated (exploring, motionless, grooming) to the learning task were also recorded and quantified. Animals were chronically implanted with stimulating and recording electrodes in hippocampal, prefrontal, and subcortical sites (n = 14) putatively relevant to the acquisition of this learning task. Field postsynaptic potentials were evoked during the performance of the above mentioned behaviors and across the whole (i.e., before, during, and after) acquisition process. Afferent perforant pathways to the hippocampus, and the intrinsic hippocampal circuit (with the exception of CA3-CA1 synapses) where eventually modified in synaptic strength during the performance of the above-mentioned behaviors. CA3-CA1 synaptic weights were only changed when learning have already acquired asymptotic values and during the performance of appetitive (i.e., going to the lever) and consummatory (i.e., eating the pellet) behaviors. In contrast, afferent (hippocampal) and efferent (accumbens septi, basolateral amygdala and reuniens nuclei) circuits of the medial prefrontal cortex where significantly modified in synaptic strength across training sessions, mostly at the moment of the largest change in the learning curve. Performance of behaviors non-directly related to the acquisition process (i.e., exploring, grooming) also evoked changes in synaptic strength at prefrontal-related circuits. Results indicate the specific and timed modification in synaptic weights of different cortical and subcortical circuits during the acquisition of an associative learning task, involving behaviors related and un-related to the learning situation.
BFU2014-56692-R

Disclosures: J. Delgado-Garcia: None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

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Program#/Poster#: 451.05/HHH26

Topic: G.01. Appetitive and Aversive Learning

Support: Gayle and Ben Batey Neuroscience Fund

Student Research Scholars program, CSUB

Title: Early exposure to a high-fat diet negatively impacts learning and memory in female rats and is ameliorated by enriched environments

Authors: *A. K. SUTER, S. HUSSAIN, N. RAMIREZ, C. DAWSON, S. MOMI, S. VILLARREAL, L. FINK, A. HUSSAIN, M. CHAUDHRY, I. C. SUMAYA;
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Abstract: The positive impact that enriched environments have on laboratory rodents has been well established. Among other outcomes, rats kept in enriched environments have consistently shown improvements in learning and memory. Because the underlying mechanism of action of learning improvements is thought to be hippocampal neurogenesis, important to our research are the emerging data showing a reduction in neurogenesis in rats fed a high fat diet. Corroborating these findings, we found high fat diets to negatively impact spatial learning in male rats and used enriched environments to ameliorate these effects (Sumaya et al., 2014). Because there have been reports showing a high fat diet affects neurogenesis differentially between male and female rats, the aim of this research was to investigate the long-term effects of a high fat diet early in development on learning and memory in females kept in enriched environments. Female rats were randomly placed in either standard cages (SC) or enriched environments (EE) at 2 mo of age and then were fed either Regular Chow (RC: 11% fat) or a Western Diet (HF: 39% fat, 44% carbs, 17% protein kcal, Modified AIN-93G, Research Diets) for 55 days. We then measured spatial learning errors in the 8-arm radial maze at 4, 10, 16, and 24 mo of age. Errors in the 8-arm maze task (averaged over the 8 days: 1 trial per day) were significantly greater in the HF group having its most detrimental effects in the groups that were housed in SC at 4 mo of age (HF: 7.80±1.07 vs. RC: 3.13±0.63 errors). At 10 mo of age, rats kept in SC and fed a HF diet did not differ on errors as compared to their controls on RC. And, regardless of diet and age, the rats housed in EE showed the least amount of errors at all testing points (RC at 4 mo: 3.13±0.63; 10 mo: 1.44±0.41 errors) (HF at 4 mo: 4.80±0.62; 10 mo: 2.20±0.50 errors). Similarly, at both 16 and 24 mo, the rats kept in SC and fed a HF diet did not differ on errors as compared to their controls on RC. And, again regardless of diet and age, the rats housed in EE showed the least amount of errors at all testing points (RC at 16 mo: 1.45±.25; HF: 2.1±0.32 errors, RC at 24 mo: 0.82 ±.28; HF: 1.33±0.24 errors). These data provide first time evidence that 1) increases in fat

intake contributes to deficits in female rodent learning and memory at the early developmental stages, and 2) enriched environments can counteract the negative effects of a high fat diet on female rodent learning and memory.

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Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

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Program#/Poster#: 451.06/HHH27

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA SC1DA034995

Title: The effect of extinction on the specific and general forms of the Pavlovian-instrumental transfer (PIT) effect.

Authors: *D. E. ALARCON, A. R. DELAMATER;
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Abstract: In the Pavlovian-instrumental transfer (PIT) effect a conditioned stimulus (CS), cue that signals a motivationally significant outcome, increases instrumental performance. Studies suggest that PIT can take a *specific* or a *general* form. In the specific, CS presentations increase responding if the CS and the responses were paired with the same outcome. But in the general form, CS presentations increase performance if the CS and responses were paired with an outcome from the same motivational valence, even though they have different sensory properties.

Research on the neural mechanisms of PIT indicate that both forms are determined by different mechanisms. For example, different regions of the amygdala and nucleus accumbens contribute to each of these forms. In rats with lesions in the basolateral amygdala or accumbens shell, the specific but not the general PIT is abolished. But in rats with lesions in the central nucleus of the amygdala or accumbens core, the CS produces the specific but not the general PIT effect (Corbit & Balleine, 2005; 2011).

Studies on specific PIT indicate that it is highly resistant to extinction procedures aimed at weakening the association between the CS and outcome (Delamater, 1996). However, there are no equivalent studies examining extinction of general PIT. If different neural mechanisms are involved in both forms of PIT then it is possible that extinction treatments may affect each form

differentially.
The studies reported here assessed the effect of extinction on specific and general PIT. Rats were trained to perform one response to obtain an outcome. Then two CSs were each paired with either the same or different outcome than the instrumental response, and two other CSs were not reinforced. Then half of the animals received non-reinforced presentations of the CSs (extinction), while the remaining animals only received context exposure. After this, subjects performed the instrumental response in the presence of the CSs.
In the control group the CS paired with the same outcome as the instrumental response elevated performance (specific PIT), but this effect was larger in the extinction group. Similarly, the CS paired with a different outcome also elevated responding (general PIT), which was more evident in the extinction group. The specific PIT was larger than the general, but both forms were reduced at a similar rate across multiple repeated PIT tests. The results suggest that extinction did not undermine either specific or general PIT effects, and, indeed, enhanced the expression of these effects, most likely by eliminating competing goal tracking CRs. Like specific PIT, general PIT also shows strong resistance to the effects of extinction.

Disclosures: D.E. Alarcon: None. **A.R. Delamater:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.07/HHH28

Topic: G.01. Appetitive and Aversive Learning

Title: Model of dopamine neurons processing temporal difference errors

Authors: *D. R. SCHUWEILER, P. A. GARRIS;
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Abstract: Temporal difference errors (TDEs) bidirectionally signal the differences between predicted and actual rewards and are used by the temporal difference learning algorithm (TDL) to update predictions about future rewards, *i.e.*, learn. Positive TDEs occur when actual events are better than predicted, *e.g.*, in response to unpredicted rewards and reward-associated cues. Negative TDEs occur when events are worse than predicted, *e.g.*, when predicted rewards or cues do not occur. A well-accepted hypothesis of mesolimbic dopamine (DA) neuron function posits that these neurons encode TDEs as the amplitude of phasic changes in action potential firing and transmit these signals to target neurons as the amplitude of phasic changes in extracellular DA. Mesolimbic DA neurons have a basal firing rate, ≈4 Hz, that briefly increases to ≈40 Hz when an animal encounters unpredicted rewards and reward-associated cues. This

slow basal rate potentially challenges the hypothesis that DA neurons bidirectionally encode TDEs, because there is a floor effect for the firing rate decrease caused by negative TDEs. Electrophysiological evidence suggests that, unlike positive TDEs, negative TDEs may alternatively be encoded by the duration of a pause in action potential generation. Neurochemical evidence suggests, however, that both positive and negative TDEs are signaled by the amplitude of a phasic change in extracellular DA. It has yet to be determined if asymmetric encoding of TDEs by DA neuron firing could cause bidirectional DA signaling of TDEs to target neurons. To determine if the changes in DA neuron action potential firing caused by TDEs could also cause bidirectional changes in extracellular DA we conducted a computational modeling experiment. We functionally coupled a TDL model to a model of DA neuron action potential generation, and in turn, a model of extracellular DA. Our models reveal that encoding of negative TDEs by pause duration, but not amplitude, is sufficient for bidirectional changes in extracellular DA. Thus, TDL remains a viable framework for understanding the function of mesolimbic DA neurons. Surprisingly, our models reveal that the amplitude of the reduction in extracellular DA while no action potentials are generated is not increased by greater DA uptake, but instead by greater action potential-dependent DA release. This paradoxical result is due to the effects of DA release increasing, and uptake decreasing, basal extracellular DA combined with the concentration-dependency of DA uptake. These results highlight the utility of our models for understanding the complex regulation of DA signals and generating novel predictions about reinforcement learning.

Disclosures: D.R. Schuweiler: None. **P.A. Garris:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.08/HHH29

Topic: G.01. Appetitive and Aversive Learning

Title: Altered metabolism but no change in working memory in rats subjected to a high-fat/high-sucrose diet

Authors: *H. M. MURPHY[1], C. H. WIDEMAN[2];
[1]Dept Psychol, [2]Biol., John Carroll Univ., Cleveland, OH

Abstract: For over thirty years, diets consisting of high fats and sugars have contributed to increased rates of obesity, type 2 diabetes, and metabolic syndrome. In addition, studies have found that they are associated with impaired cognition. The present study investigated the effects of a high-fat/high-sucrose (HFHS) diet on body weight, caloric intake, blood glucose levels, adiposity, spatial working memory, and anxiety-like behavior. Ten female rats were habituated

to a control diet for one week. Subsequently, the rats were divided into two groups: a high-fat/high sucrose (HFHS) diet group and a control diet group. This experimental phase continued for four weeks. During the last week of the experiment, a Morris Water Maze (MWM) was utilized to study trial-dependent spatial working memory. All maze sessions were conducted during the dark phase of the circadian cycle utilizing red light. Each rat was given one session consisting of two trials each day. The first was the sample trial in which the animal discovered the location of the platform by trial and error and the second was the test trial in which the animal was required to recall the location of the platform. Time to reach the platform was recorded in each trial. During the sample trial, the animal was given 90s to find the platform. After locating the platform, the animal was allowed to rest on the platform for 15s. If the animal did not find the platform within the 90s period, it was guided to the platform and allowed to rest for 15s on the platform. Following the rest period, the animal was picked up and put back into the starting location and given 90s to find the platform in the test trial. The platform was relocated every day. Because of relocation, no learning of the platform position from the previous day could be transferred to the test trial of the next day; hence, recall on each day during the test trial was dependent on the sample trial of that day and measured only temporary or working memory. During all MWM sample and test trials, anxiety was rated on a scored scale of 0 - 3 by assessing rat behavior prior to being lowered into the water maze. At the conclusion of the experiment, blood glucose levels and renal and mesenteric adiposity were measured. The current study found that rats on the HFHS diet had significantly greater: percent increase in body weight, daily caloric intake, blood glucose concentration, and adiposity. No significant difference was found between groups in terms of spatial working memory. A noteworthy finding, however, was that HFHS rats showed a significant increase in anxiety-like behavior between successive MWM trials. Further research is needed as the popularity of the HFHS diet continues to increase.

Disclosures: H.M. Murphy: None. **C.H. Wideman:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.09/HHH30

Topic: G.01. Appetitive and Aversive Learning

Support: W911NF-14-2-0045

Title: Neural correlates of decision making in the aversive-reward conflict task

Authors: *A. AFZAL[1], S. ZOROWITZ[1], K. K. ELLARD[1], A. S. WIDGE[1], A. GILMOUR[1], D. DOUGHERTY[1], E. ESKANDAR[2], T. DECKERSBACH[1];
[1]Psychiatry, [2]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: The psychological domain of Approach-Avoidance attempts to capture an individual's behavioral response to appetitive and aversive stimuli in the environment to measure how individuals weigh the threat of aversion against the possibility of reward. To study the functional neurobiology of approach-avoidance behavior in healthy individuals, we have developed a new experimental paradigm, the Aversive-Reward Conflict (ARC) task, that integrates both risk and reward.
Methods: 21 healthy volunteers performed the fMRI task In this task, participants choose between a high reward ($0.06 - $0.96) combined with a specific probability (low, medium, high) of electric stimulation and a low reward ($0.01) with no probability of electric stimulation. As such, participants have the option to completely avoid an aversive experience in every trial. We employed a Bayesian regression model to estimate a per-trial decision boundary which was entered as parametric modulation into fMRI analysis.
Results: Group level analysis of trials at the decision boundary shows bilateral activation in dorsolateral prefrontal cortex, dorsal anterior cingulate , posterior cingulate and insula. Analysis for the slope term, showing modulation from the decision boundary, shows deactivation in dlPFC, dACC, PCC and insula. Analysis of risk and reward conditions show greater activation in left anygdala for high risk and caudate and putamen for high reward, respectively.
Conclusions: The results highlight the important interactions between reward valuation and cognitive control systems during simultaneous risk and reward processing. Increased activation in the above regions for high conflict trials and decreased activation for low conflict trials confirm previous findings about the role of the dlPFC, dACC and insula in the processing of conflicting stimuli during decision making. Negative correlations between reward and BAS scores for high risk show that individuals with lower appetitive dispositions require a higher reward to pursue the risky option. This result shows that the ARC task effectively taps into the approach-avoidance domain in creating response conflict.

Disclosures: A. Afzal: None. **S. Zorowitz:** None. **K.K. Ellard:** None. **A.S. Widge:** None. **A. Gilmour:** None. **D. Dougherty:** None. **E. Eskandar:** None. **T. Deckersbach:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.10/HHH31

Topic: G.01. Appetitive and Aversive Learning

Title: Rewarding stimulation of the medial prefrontal cortex activates extensive brain regions: an optogenetics-fMRI study

Authors: *Y. HU, A. TALISHINSKY, H. LU, S. IKEMOTO, Y. YANG;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Recent deep brain stimulation (DBS) study in major depression patients suggested an important role of white matter bundles connecting medial prefrontal cortex (MPFC) in treatment efficacy. Animal research suggests this region in positive emotion or reward. To better understand brain circuits responding to the stimulation, in the present study, we incorporated optogenetics and functional magnetic resonance imaging (fMRI) to investigate MPFC-mediated behavior. Channelrodopin2-expressing adeno-associated virus (AAV-ChR2) or control virus (AAV-eYFP) was injected into the ventral MPFC (vMPFC) in two groups of rats (experiment n=15, control n=7), respectively. Animals were trained to press lever for optogenetics stimulation (a 25-Hz train of 8 pulses). All animals then underwent fMRI scanning in a Bruker Biospin 9.4T scanner, keeping anesthetized with a combination of isoflurane (0.5%) and dexmedetomidine hydrochloride (0.015 mg/kg/hr). Stimulation trains were delivered in a block-design pattern with 20s stimulus on and 40s off. FMRI data were acquired using a T2*-weighted EPI sequence (TE/TR = 13/1000 ms, TR = 1000 ms, segment = 2, FOV = 35 × 35 mm^2, matrix size = 64×64, slice thickness = 1 mm, slice number = 15). Brain activation and its relationship with rewarding behavior were examined. Stimulation of vMPFC activated many areas along known projections of the MPFC. Specifically, the entire MPFC, including the medial orbital, prelimbic, and infralimbic regions were activated. The stimulation also activated cortical and subcortical regions that receive the vMPFC afferents, including the agranular insular area, anterior cingulate area 2, tenia tecta, amygdala, ventral striatum, septal area, bed nucleus of stria terminalis, midline anterior thalamic areas, preoptic area, and hypothalamus. Lever press behavior was positively correlated with brain activation of hypothalamus ($r=0.76$, $p=0.01$), anterior insula ($r=0.69$, $p=0.028$) and ventral striatum ($r=0.64$, $p=0.047$). Our study revealed that stimulation of the vMPFC activates extensive brain regions, and some of the activation was correlated with the lever press behavior. The vMPFC corresponds to the subcallosal cingulate, which has been an effective target of deep brain stimulation for the treatment of depression. Our finding may shed light on circuits involved in therapeutic effects of DBS. This research was supported by the Intramural Research Program of the NIH, NIDA

Disclosures: **Y. Hu:** A. Employment/Salary (full or part-time): This research was supported [in part] by the Intramural Research Program of the NIH, NIDA. **A. Talishinsky:** None. **H. Lu:** None. **S. Ikemoto:** None. **Y. Yang:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.11/HHH32

Topic: G.01. Appetitive and Aversive Learning

Support: BFU2014-56692-R

Title: Functional states of hippocampal and prefrontal circuits characterizing the acquisition of an operant conditioning tasks and related and unrelated behaviors in alert behaving rats

Authors: *A. GRUART;
Pablo de Olavide Univ., Seville, Spain

Abstract: A general assumption of contemporary neuroscience is that learning is stored in the brain in the form of more-or-less stable changes in synaptic strengths in selected cortical/subcortical sites. Nevertheless, a still-open question is whether synapses included in hippocampal and medial prefrontal circuits, present similar changing rates in synaptic efficacy during the acquisition of operant conditioning tasks in behaving rats. In the present study, we have recorded activity-dependent changes in synaptic strength —namely, the slope of the chronically evoked field postsynaptic potentials (fPSPs) in 13 selected synapses: perforant path (PP)-dentate gyrus (DG), PP-hippocampal CA3 area, PP-hippocampal CA1 area, DG-CA3 rostral, DG-CA3 caudal, CA3-contralateral CA1, CA3-ipsilateral CA1, CA1-thalamic reuniens nucleus (REU), CA1-subiculum, CA1-medial prefrontal cortex (mPFC), mPFC-basolateral amygdala, mPFC-nucleus accumbens septi, and mPFC-REU. Changes in synaptic strength evoked at the mentioned synapses were recorded during behaviors related (approaching to the lever, lever pressing, approaching the feeder, eating) and unrelated (exploring, motionless, grooming) to the acquisition process. We characterized two types of evolution patterns: (1) synaptic-timing states during each selected behavior; and, (2) behavioral-timing states related to each synapse. Results indicate that whereas the synaptic strength in the main inputs and in the intrinsic synapses of the hippocampal circuit do not change significantly along the operant conditioning, the synaptic strength in the main outputs (except for CA1-SUB) and in the extrinsic synapses increases significantly in parallel with the acquisition rate of the associative learning task. Furthermore, these dynamical changes were similar for the different types of behavior (locomotor, stationary, appetitive, consumatory, exploratory) considered here. Thus, the precise and timed activation of multiple synaptic contacts during an operant conditioning task evoked a definite and specific dynamic map of synaptic states characterizing behaviors related and unrelated to the learning process.
BFU2014-56692-R

Disclosures: A. Gruart: None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.12/HHH33

Topic: G.01. Appetitive and Aversive Learning

Title: Methylphenidate shifts healthy volunteers towards a reward-sensitive strategy during learning-based decision-making

Authors: *K. M. HARLE[1], C. HYSEK[2], S. ZHANG[3], A. YU[2], M. PAULUS[2];
[1]Dept. of Psychiatry, UCSD, La Jolla, CA; [2]UCSD, San Diego, CA; [3]UCSD, Cognitive Science, CA

Abstract: ***Introduction***: Computational approaches such as Bayesian ideal observer models have the potential to more powerfully and accurately delineate the cognitive dysfunctions associated with psychiatric disorders, as well as the improvement of such dysfunctions with pharmacological or behavioral treatments. Having recently observed decision-making abnormalities in occasional and dependent stimulant users with such methods, here we use a similar Bayesian modeling approach to quantify the impact of a common prescription stimulant, methylphenidate (MPH), on exploratory reward-based decision-making.
Methods: 24 healthy volunteers received an acute oral administration of placebo or 40 mg MPH (1 month apart) and after about 120 minutes completed 20 games of a 3-armed bandit task. In each game, they repeatedly choose between three arms with fixed but unknown reward rates, with the goal of maximizing total reward. The task was formalized as comprising a learning component, which updates estimated reward rates based on ongoing observations (modeled by iterative Bayesian inference), and a strategic component, which choses among options based on current expectations about reward rates (modeled by 4 decision policies: Win-stay/Lose-shift (WSLS), ε-Greedy, τ-Switch, Softmax). **Results:** Significantly more individuals were best fit by a learning-based Softmax strategy than by a learning-independent WSLS strategy during MPH administration ($p<.05$). For Softmax users, task completion under MPH administration was associated with a higher reward sensitivity bias (Beta=4.99), i.e., higher propensity to choose the option estimated to be most rewarding, relative to completing the task under placebo (Beta=3.39, $p=.02$). Softmax parameter under MPH administration was further positively correlated with monetary gains in the task ($r=.64$, $p<.05$).
Conclusions: MPH may promote utilization of a more reward-sensitive strategy and higher reward responsiveness in learning-based decision-making. MPH has recently been found to enhance extinction learning; this study shows that it may also affect reward-based learning. The computational approach helps to better delineate how MPH changes learning strategies and can be used to provide a rationale for this or similar drugs to be used in psychiatric disorders that show significant learning dysfunctions such as ADHD and depression.

Disclosures: **K.M. Harle:** None. **C. Hysek:** None. **S. Zhang:** None. **A. Yu:** None. **M. Paulus:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.13/HHH34

Topic: G.01. Appetitive and Aversive Learning

Support: NIDDK R01 DK085721

Title: The effects of chemogenetic inactivation of the medial prefrontal cortex during Pavlovian appetitive conditioning

Authors: ***S. E. KEEFER**, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Learned environmental cues that predict food can override internal, physiological signals and stimulate eating in the absence of hunger. The medial prefrontal cortex (mPFC) is recruited during cue-food associative learning and is necessary for overeating in the presence of these learned cues. However, less is known about the role of the mPFC across different stages of learning. The current study examined whether the mPFC is necessary during the initial and well-learned stages of Pavlovian cue-food acquisition by using DREADDs (designer receptors exclusively activated by designer drugs), a chemogenetic method of neuronal inactivation. Male, Long-Evans rats received bilateral injections into the mPFC of an adeno-associated virus (AAV) expressing inhibitory DREADD (AAV5-hSyn-HA-hM4D-IRES-mCitrine) in order to selectively silence mPFC neurons during specific conditioning sessions. Rats underwent six sessions of Pavlovian appetitive conditioning, one session per day. Each conditioning session included 8 presentations of a 10s tone (conditioned stimulus, CS) follow by immediate delivery of 2 palatable food pellets (unconditioned stimulus) distinct from standard chow. Thirty minutes prior to session 3 (early learning) and session 6 (well-learned), rats received an i.p. injection of either clozapine N-oxide (CNO; biologically inert, DREADD-selective ligand, 3 mg/kg) or vehicle. Preliminary results indicate that during conditioning session 3, inactivation of the mPFC decreased overall motivation to approach the food cup, measured as percentage of time rats spent expressing food cup behavior (conditioned response) during the 10s prior to the onset of the CS (pre-CS), during the CS, and during the 10s after the offset of the CS (post-CS) when food pellets are available. Even though there was an overall decrease to approach the food cup throughout session 3, inactivation of the mPFC resulted in a more selective responding during the CS in the second half of the session, as indicated by an elevation score (CS minus pre-CS

responding). No significant differences were found during conditioning session 6, suggesting the mPFC may not be essential in the expression of well-learned cue-food associations. These results indicate a role for the mPFC in Pavlovian appetitive conditioning, specifically during the initial motivation to learn cue-food associations.

Disclosures: S.E. Keefer: None. **G.D. Petrovich:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.14/HHH35

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01DK085721

Title: DREADD inactivation of medial prefrontal cortex neurons disrupts renewal of Pavlovian conditioned responding to food cues in male rats

Authors: *L. C. ANDERSON, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Cues associated with food can stimulate appetite and food consumption independently of hunger and this non-homeostatic eating is a contributor to the rise in obesity in our society. Renewal, or reinstatement, of responding to food cues after extinction may explain the inability to resist palatable foods and change maladaptive eating habits. Recently, we examined recruitment (Fos induction) of the medial prefrontal cortex during context-dependent renewal in male rats. We found higher Fos induction within the infralimbic and prelimbic regions of the ventromedial PFC (vmPFC) in rats exhibiting renewal of responding compared to a control group. Here, we examined whether the vmPFC is necessary during renewal using the chemogenetic methodology DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to silence vmPFC neurons during the test for renewal. Male, Long-Evans rats received bilateral stereotaxic injections into the vmPFC of a viral vector containing the gene for a synthetic inhibitory G-protein-coupled receptor (AAV5-hSyn-HA-hM4D-IRES-mCitrine) or a control viral vector (AAV5-hSyn-EGFP). After recovery rats were trained in a within-subjects Pavlovian context-dependent renewal protocol. Rats were trained to associate a tone (conditioned stimulus, CS) with food (unconditioned stimulus) in 5 acquisition sessions (one session per day) in Context A. Acquisition was followed by 2 extinction sessions with CS-only presentations in Context B. Rats were tested for renewal of responding with CS-only presentations in Context A and Context B, counterbalanced for order, on separate days. 30 minutes prior to each test, rats

were injected with clozapine N-oxide (CNO, biologically inert, DREADD-selective ligand; 3mg/kg, i.p.) or vehicle. Rats either received CNO on both days or vehicle on both days. The resulting groups were: DREADD+CNO, DREADD+Vehicle, and Control Virus+CNO. The measure of learning was an increase in the expression of food cup behavior (conditioned response, CR) during CSs. Renewal of responding was determined by significantly higher CRs in the acquisition context (Context A) compared to the extinction context (Context B). There was a main effect of Context and Group interaction in a Repeated Measures ANOVA ($p<0.05$). *Post hoc* tests revealed the DREADD+CNO group failed to show higher CRs in Context A compared to B. Both the DREADD+Vehicle and Control Virus+CNO groups exhibited higher CRs in Context A. Silencing vmPFC neurons with DREADDs disrupted renewal of food cup responding, indicating that the vmPFC is critical in renewal of Pavlovian conditioned responding to food cues.

Disclosures: L.C. Anderson: None. **G.D. Petrovich:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.15/HHH36

Topic: G.01. Appetitive and Aversive Learning

Support: Fonds de recherche du Québec – Santé

CIHR

Concordia University

Title: The effect of intermittent versus continuous training on the incentive salience of a Pavlovian alcohol cue

Authors: *F. R. VILLARUEL, S. HEFFERNAN, M. CHAHINE, N. CHAUDHRI; Concordia Univ., Montreal, QC, Canada

Abstract: Pavlovian cues that predict alcohol can support sign-tracking and function as conditioned reinforcers, indicating that they acquire incentive salience. Moreover, intermittent alcohol exposure can facilitate alcohol intake and preference. We hypothesized that an intermittent schedule of Pavlovian training would also promote the attribution of incentive salience to a conditioned stimulus (CS) that predicted alcohol.
Male, Long-Evans rats were acclimated to 15% ethanol (EtOH) in the home-cage and then divided into two groups, one that received training every weekday (Monday to Friday), and a

second that received training on alternate days of the week (including weekends). Both groups received 27 Pavlovian conditioning sessions. In each session, a retractable lever-CS (10 s) was paired with the delivery of EtOH (0.2 mL per lever-CS; 12 trials per session) into a fluid port for oral consumption.

Rats rapidly learned to approach the fluid port during presentations of the lever-CS, referred to as a 'goal-tracking' conditioned response. Gradually however, goal-tracking responses diminished as rats started approaching and interacting with the lever-CS (referred to as a 'sign-tracking' conditioned response). Interestingly, from sessions 10 to 18 sign-tracking was augmented in rats that received intermittent training. Control groups that received unpaired presentations of the lever-CS and EtOH failed to develop goal- or sign-tracking responses, regardless of training schedule. Thus, the development of conditioned responding required a predictive relation between the lever-CS and EtOH.

Following Pavlovian conditioning, rats were tested in a conditioned reinforcement procedure in which nosepokes into an 'active' aperture lead to presentations of the lever-CS (2.5 s), while nosepokes into an 'inactive' aperture had no programmed consequence. Presentations of the lever-CS did not significantly enhance active nosepokes relative to inactive nosepokes. However, rats that previously received paired presentations of the lever-CS and EtOH exhibited more sign-tracking responses when the lever-CS was presented as a result of active nosepokes, compared to rats in unpaired groups.

Thus, the schedule of Pavlovian training can influence the attribution of incentive salience to an alcohol cue as measured by sign-tracking. Future experiments will compare continuous Pavlovian training (everyday) with an alternate day schedule; examine the extinction and reinstatement of sign-tracking and goal-tracking; and investigate the impact of different schedules of reinforcement on responding with conditioned reinforcement.

Disclosures: F.R. Villaruel: None. **S. Heffernan:** None. **M. Chahine:** None. **N. Chaudhri:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.16/HHH37

Topic: G.01. Appetitive and Aversive Learning

Support: PF

IUAP

FWO Flanders

HBP

Title: Simple paradigm for investigating the learning of cost-benefit associations in monkeys.

Authors: T. VANDE CASTEELE[1], J. ARSENAULT[1,2], *W. VANDUFFEL[3,1,2];
[1]K.U. Leuven, Leuven, Belgium; [2]Athinoula A. Martinos Ctr. for Biomed., Mass. Gen. Hosp., Charlestown, MA; [3]Radiology, Harvard Med. Sch., Charlestown, MA

Abstract: In most instances, before a reward can be consumed effort must be expended to acquire it. Converging behavioral data show that when subjects make cost-benefit decisions, they weigh the value of potential rewards against the perceived costs of their effort. Studies have linked dopaminergic (DA) signaling to these calculations. For instance, while response properties of primate DA neurons reflect the value of cues associated with reward (Schultz et al., 1997), these responses are reduced when the effort associated with a cue is increased (Varazanni et al., 2015). In addition to DA neurons themselves, their target sites like the striatum and prefrontal cortex, are also critical in cost-benefit processing (Friedman et. al., 2015). With the advent of techniques allowing for the reversible perturbation of specific pathways within primates (Kinoshita et al., 2011), it is now possible to causally investigate the role of specific neural circuits within the primate in cost-benefit processing. To help investigate this, we aimed to develop a simple behavioral task to examine cost-benefit behavior in primates, with the primary goals that the task: 1) was easy to learn, 2) demonstrated cost-benefit decision making, 3) exhibited learning of new cost-benefit associations, 4) was affected by manipulations in reward magnitude (a surrogate for DA signaling). Therefore we designed a paradigm in which, after fixating on a central point (500 ms), monkeys were given a choice between 2 visual cues. Cues were positioned randomly in 2 of 4 possible positions to avoid spatial response biases. To select a cue, monkeys had to saccade to this cue and maintain fixation for a period of time. The duration of fixation determined the effort level (cue A = 1 s; cue B = 5 s; cue C = 9 s) and juice amount determined the reward level. To manipulate reward magnitude, we ran one variant of this paradigm with lower reward values (cue A = 0.11 ml; cue B = 0.54 ml; cue C = 0.98 ml) and the other with higher reward values (cue A = 0.16 ml; cue B = 0.81 ml; cue C = 1.46 ml). For each session 3 new visual cues were used and a randomized pair of cues were displayed on a given trial. We found that 1) animals were able to quickly learn this task, 2) during all sessions cue B became the preferred cue demonstrating a trade-off between the low effort cue (cue A) and the high reward cue (cue C) -possibly reflecting a preference for 'middle options' as seen in humans (Rodway et al., 2011), 3) preference for cue B increased during a session, 4) preference for cue B increased more quickly during higher reward sessions. These initial behavioral experiments pave the way for future studies investigating the causal role of specific pathways in cost-benefit processing.

Disclosures: **T. Vande Casteele:** None. **J. Arsenault:** None. **W. Vanduffel:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.17/HHH38

Topic: G.01. Appetitive and Aversive Learning

Support: Korea Research Foundation Grant (NRF-2014R1A1A2058480)

Title: Lateral habenula plays a role in the process by which no association between two events is learned

Authors: *D.-H. KIM, B.-R. CHOI, J.-S. HAN;
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Abstract: In the reward conditioning, midbrain dopaminergic system (DA) is activated by reward delivery while lateral habenula (LHb), which known as major inhibitory center of DA system, is working in opposite manner with DA system. Previous studies have showed that DA system is involved in Pavlovian appetitive conditioning. However, no study has done to reveal a role of LHb in Pavlovian appetitive conditioning. Therefore, the present study was conducted to examine the role of LHb in Pavlovian appetitive conditioning. In a paired group, light was presented for 10 sec and immediately delivered two reward pellets, and 8 trials per one session for 64 min with 8 min variable inter-trial interval and conditioning was performed for 8 days. In an unpaired control group, the same number of lights and food pellets were pseudorandomly presented using an unpaired procedure in which the CS and US were non-contingent. And in a truly random control (TRC) group, presented 4 paired light-food and 8 unpaired stimuli (4 unpaired lights and 4 unpaired foods) with pseudo-random order. Three control groups (light or food only received group and naïve group) were included to exclude chance that alterations of neural activity by stimuli presentation and apparatus exposure. We measured two conditioned responses (CR), food-cup response and orienting response. Both CRs were increased in paired group, but not in unpaired group. And theses conditioned responses in TRC group were increased, but these response levels were an intermediate point between levels of paired group and levels of unpaired group. Next, we measured the neuronal activity by c-fos expression levels with immunohistochemistry in LHb and other brain area, substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) and basolateral amygdala (BLA), central amygdala (CeA). LHb neuronal activity was only increased in unpaired group, but, was not increased in paired and TRC groups. And, neuronal activity of other brain areas (SNc, VTA, BLA and CeA) was increased in paired group, but not in unpaired group. Additional experiment using the retrograde tracer cholera toxin B subunit revealed that these c-fos expressed neurons in LHb were projected to SNc or VTA. Most c-fos expression neurons in unpaired group were also projected to SNc or VTA. These results suggest that the LHb play a role in the process by which no association

between two events is learned, in opposition to the midbrain dopaminergic neurons involved in the process by which an association between two events is learned. Supported by the Korea Research Foundation Grant funded by the Korean Government (NRF-2014R1A1A2058480) to Jung-Soo Han

Disclosures: **D. Kim:** None. **B. Choi:** None. **J. Han:** None.

Poster

451. Appetitive and Incentive Learning and Memory: Conditioning I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 451.18/HHH39

Topic: G.01. Appetitive and Aversive Learning

Support: NIH P60 AA011605

NIH P60 AA011605-S1

NIH T32 AA007573

Title: Nicotine enhances Pavlovian conditioned responding in male and female rats

Authors: *S. J. STRINGFIELD[1,2], A. C. MADAYAG[2], J. XU[2], C. A. BOETTIGER[2,3], D. L. ROBINSON[1,2];
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Abstract: In Pavlovian conditioned approach behavior, animals often demonstrate the conditioned responses (CRs) of sign tracking and goal tracking in which they approach and interact with a conditioned cue or the location of reward delivery, respectively. Sign tracking is associated with the attribution of incentive salience to the conditioned cue and has been linked to multiple measures of addiction vulnerability. Nicotine administration during Pavlovian conditioning increases the likelihood that an animal will display a CR. Sex differences in CRs have been described in animal models, with females sometimes exhibiting more sign tracking. Sex differences in epidemiological studies of male and female smokers have been reported, which are supported by sex-dependent effects in animal models of nicotine reinforcement. We aimed to measure sex differences in the nicotine-induced enhancement of CRs, either due to sex differences in inherent biological factors or in the effect of nicotine on the development and expression of this behavior. We hypothesized that nicotine would enhance CRs in both sexes, and that nicotine-exposed females would show even greater sign tracking than would males. To test this hypothesis, adult male and female Sprague Dawley rats were trained to associate a 30 s

presentation of a compound light and lever cue with subsequent delivery of a sucrose reward over 29 training sessions, each consisting of 15 cue-reward pairings. Rats in the nicotine exposure group received 0.4 mg/kg nicotine SC prior to each session, and a control group received saline injections. We report that nicotine enhanced both sign tracking and goal tracking CRs in both males and females, with females showing faster CRs, but no interaction between sex and nicotine was found. On measures of sign tracking, nicotine-exposed rats exhibited decreased latency to press the lever, and increased lever presses and probability of pressing the lever. For measures of goal tracking, nicotine-exposed rats showed an increase in receptacle elevation score (i.e., receptacle entries during the cue minus entries before the cue). Females displayed CRs faster than males, as indicated by a main effect of sex on latency to press the lever and an interaction of sex by day on latency to enter the receptacle. Thus, we replicate previous findings indicating that nicotine enhances conditioned responding, and extend this effect to females. In addition, we identified sex differences in the expression of these CRs. Future studies will use this cohort of male and female rats to investigate putative differences in gene expression correlated with both nicotine exposure and the expression of CRs.

Disclosures: S.J. Stringfield: None. **A.C. Madayag:** None. **J. Xu:** None. **C.A. Boettiger:** None. **D.L. Robinson:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Topic: G.01. Appetitive and Aversive Learning

Support: NIDA DA025922

T32 NIAAA 5T32AA007468-28

DOD W81XWH-12-2-0048

ARCS Roche Scholar

Title: Characterization of rapid reacquisition of contextual fear behavior following post-extinction reconditioning in C57BL/6J mice and Long Evans rats

Authors: *A. WILLIAMS[1], K. M. LATTAL, 97210[2];
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Abstract: Associative learning is mainly studied from the perspective of initial conditioning, when new associations are established, or extinction, when those associations are inhibited. Although extinction eliminates behavior, it does not eliminate the original association. One piece of under-studied evidence for this is rapid reconditioning or reacquisition; very brief conditioning episodes (defined as 'reconditioning') can restore the behavior that is eliminated during extinction. The behavioral model used here allows for direct comparison of contextual fear acquisition and reacquisition in two species of rodents. We find that following moderate extinction, rodents display a persistent and rapid reacquisition of fear behavior relative to animals being conditioned for the first time. Following massive extinction, C57BL/6J mice do not rapidly reacquire fear following reconditioning, but Long Evans rats display rapid reacquisition regardless of the amount of extinction prior to reconditioning. Further, we show that the rapid reacquisition of fear is context-specific and not a sensitized or reinstated response to the unconditioned stimulus (footshock) in an alternate context. Finally, preliminary inactivation studies (muscimol + baclofen microinjections) explore the role of the extended amygdala (anterior bed nucleus of the stria terminalis) in the rapid reacquisition of contextual fear. These results suggest that post-extinction reconditioning differs behaviorally from initial conditioning in that reconditioning results in the rapid reacquisition of fear behavior contingent upon the amount of extinction received and the species of rodent. Rapid reacquisition of fear behavior provides a potential behavioral correlate of post-traumatic stress disorder symptoms, such as exaggerated startle response and hypervigilance, through which to study the behavior, neurobiology, and potential therapeutic targets of specific PTSD symptoms.

Disclosures: **A. Williams:** None. **K.M. Lattal:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 452.02/III1

Topic: G.01. Appetitive and Aversive Learning

Support: Sao Paulo Research Foundation grant 2013/13665-0

Title: Delta and low theta band mediates tone fear conditioning under urethane anesthetized rats

Authors: *E. F. OLIVEIRA[1], M. B. REYES[2];
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Abstract: The Memory is a universal characteristic with high adaptive value present in vertebrates and invertebrates. Electrophysiological and behavioral evidences suggest that fear conditioning - which is related to implicit memory process - can be acquired under anesthesia. Additionally, the amigdalo-nigro-striatal pathway is assumed to play an important role in the acquistion of associative learning. Here, we ask the role of dorsal striatum in the acquisition of tone fear conditioning under anesthesia. To investigate this question, the local field potential (LFP) in the dorsal striatum of urethane-anesthetized wistar rats was recorded. We looked for changes in electrophysiological activity related to the acquisition of a fear conditioning protocol. The task consisted of 5 s two Conditioned Stimulus (CS), which could be either a Tone or White Noise randomly presented and counterbalanced. The stimuli were assigned as CS+ (paired with Unconditioned Stimulus (UCS)) or CS- (not paired with UCS). UCS where 1 mA foot-shock with 1 s duration delivered in the last 1 s of CS+. Recordings were made in 3 phases: 1) Pre-pairing: 60 presentations of both CS+ (without US) and CS-, to establish a baseline; 2) Paring: 60 pairings between CS+-UCS and 60 CS- presentations); 3) Post-pairing: 60 CS+ (without UCS) and 60 CS- presentations. Our main found is an increase in power for delta and low theta showed up for both CS+ and CS-, both in the pre-pairing and post-pairing CS presentation. Looking to the time that this increase in power lasted and subtracting the duration of post-pairing from the duration in pre-pairing periods, we found that the increase in CS+ in post-pairing was significantly longer than CS- in delta and low theta band, but not for high theta (8 - 12 Hz). Our results suggest that tone fear conditioning can be acquired under anesthesia, with delta and low theta band mediating the retrieval process. Additionally, CS+ and CS- appears to be discriminated. It is arguable that CS+ can have a transfer effect to CS-, in this case CS- should have the same electrophysiological signature as CS+, which is not the case. Therefore, we conclude that, even under anesthesia, rats can learn tone fear conditioning and discrimante CS+ and CS-.

Disclosures: **E.F. Oliveira:** None. **M.B. Reyes:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 452.03/III2

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH

Title: Basal ganglia output controls active avoidance behavior

Authors: S. HORMIGO, G. VEGA-FLORES, *M. A. CASTRO-ALAMANCOS;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Engrained avoidance behavior is highly adaptive when it keeps away harmful events and can be highly maladaptive when individuals elude harmless situations in anxiety disorders, but the neural circuits that mediate avoidance are poorly understood. We found that the output of the basal ganglia, through the substantia nigra pars reticulata (SNr), controls active avoidance. We employed chemogenetics and optogenetics to control the activity of SNr cells in a vesicular GABA transporter-IRES-Cre mouse strain. This allowed to express hM3Dq-DREADD, hM4Di-DREADD, channelrhodopsin-2 (ChR2), or a variant of archaerhodopsin -ArchT- in SNr cells. Neurons expressing hM3Dq are excited, while neurons expressing hM4Di are inhibited, after i.p. injections of clozapine N-oxide. Neurons expressing ChR2 are excited by blue light, while neurons expressing ArchT are inhibited by green light. The results show that SNr excitation blocks avoidance to a conditioned sensory stimulus while preserving the ability to escape the harmful event. Conversely, SNr inhibition facilitates avoidance to the conditioned stimulus and suffices to drive avoidance without any conditioned sensory stimulus. The results highlight a midbrain circuit that gates avoidance responses, which can be targeted to ameliorate maladaptive avoidance in psychiatric disorders.

Disclosures: S. Hormigo: None. **G. Vega-Flores:** None. **M.A. Castro-Alamancos:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 452.04/III3

Topic: G.01. Appetitive and Aversive Learning

Title: Personality traits contribute to voluntary pain-related avoidance behavior

Authors: *Y. NISHI[1], M. OSUMI[1], S. NOBUSAKO[1], K. TAKEDA[2], S. MORIOKA[1];
[1]Kio Univ., Kitakatsuragi-Gun, Japan; [2]Hokkaido Univ., Sapporo-Shi, Japan

Abstract: Introduction There are people avoiding a pain excessively in chronic pain patients. Their avoidance behaviors cause a vicious circle toward chronic pain. However, it is not revealed what kinds of personality traits are related to avoidance behaviors. Therefore, this study aimed to investigate the type of personality traits which contribute avoidance behaviors.
Method Twenty-four healthy participants performed a task to paint rectangular displayed on a touch panel with a touch pen as conditioned stimulus (CS). A trial continued 30 sec after beginning to paint. The task consisted of several phases: 5 practice trials, 5 acquisition trials, and

20 test trials which were separated into 4 blocks. Only in acquisition phase (CS+), participants were instructed to paint over 15 sec in each trial while there was no time constraint in practice phase (CS-) and test phase (CS+). The CS+ was followed by the painful electrocutaneous stimulus as the unconditioned stimulus only during painting. In each trial, auditory startle probes were performed after onset of paint and each peak amplitude were transformed to z-scores (startle responses). We calculated the time in which participants do not paint (avoidance time) at every phases. In addition, they answered State-Trait Anxiety Inventory (STAI) and revised temperament and character inventory (TCI-R) before this experiment. Participants were classified according to avoidance time using cluster analysis (Ward' method). To compare each score of STAI, TCI-R, avoidance time and startle responses among classified subgroups, t-test or Mann Whitney U-tests were used. Moreover, to compare scores of startle responses among each phase, the Friedman test was used. The Bonferroni correction was used to adjust the p-values obtained in the post hoc analyses (avoidance time; $p<0.01$, startle responses; $p<0.002$).
Results and Discussions In the result of clustering, participants were divided in two subgroups as follows; cluster 1 (n=6), cluster 2 (n=18). Startle responses of both subgroups enhanced significantly in acquisition phase compared to practice and test phases, but there were no significant differences between both subgroups. Avoidance time of cluster 1 was significantly longer than that of cluster 2 in all phases. Cluster 1 was significantly higher in STAI-trait and harm avoidance item involved in TCI-R than cluster 2. Also Cluster 1 was lower in novelty seeking item involved in TCI-R than cluster 2. Nevertheless, both subgroups were caused fear conditioning of pain in this study, they showed different pain-related avoidance behavior. Consequently, their avoidance behavior were different depending on each personality trait.

Disclosures: **Y. Nishi:** None. **M. Osumi:** None. **S. Nobusako:** None. **K. Takeda:** None. **S. Morioka:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA034010

Title: Sub-second fear discrimination in rats: Adult impairment in adolescent heavy alcohol drinkers

Authors: ***M. A. MCDANNALD**, K. M. WRIGHT, A. DILEO;
Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: The ability to accurately discriminate safety from danger is vital. However, discrimination should not only be accurate, but should also emerge rapidly following encounters. Rapid fear discrimination may be independent from the general ability to discriminate and more susceptible to adolescent heavy alcohol drinking, which increases the lifetime risk for post-traumatic stress disorder. Despite its importance, we unaware of any study assessing the rapidity with which fear discrimination emerges following cue onset in any mammal. Here we sought to uncover the normal rapidity of fear discrimination and its possible impairment in adolescent heavy alcohol drinkers in rats. We gave rats voluntary access to alcohol throughout adolescence and identified heavy drinkers. In adulthood, we assessed rapid fear discrimination in a paradigm in which three cues were associated with three different probabilities of shock: safety p=0.00, uncertainty p=0.25 and danger p=1.00. Normal rats readily acquired sub-second fear discrimination, showing differential fear to safety, uncertainty and danger cues ~450-550 ms following onset. Adolescent heavy alcohol drinkers were impaired in sub-second fear discrimination, with discrimination not emerging until ~2100-2600 ms following cue onset. The deficit in adolescent heavy drinkers was not a general learning decrement, as these rats showed excellent discrimination later in the cue. These results provide the first evidence of sub-second fear discrimination in rats. Further, these results mark rapid fear discrimination as an ability critical to normal function and relevant to post-traumatic stress disorder.

Disclosures: **M.A. McDannald:** None. **K.M. Wright:** None. **A. DiLeo:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Program#/Poster#: 452.06/III5

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01HD075066

Title: Optogenetic analysis of prefrontal contributions to contextual fear memories

Authors: ***A. ASOK**[1], D. V. GAGLIARDOTTO[2], A. M. HUGHES[2], J. SCHULKIN[3], J. B. ROSEN[2];
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Abstract: The medial prefrontal cortex (mPFC) has received considerable attention for its role across different phases of fear-learning and memory, particularly expression and extinction. While there has been substantial progress in understanding how the mPFC regulates fear to

discrete cues (e.g., tones and lights), less is known about the mPFC's involvement in learning fear to a context. Therefore, the present study examined the effects of mPFC inhibition during acquisition on the long-term retention and extinction of contextual fear. Rats received bilateral infusions of a pan-cellular optogenetic neural silencer CAG-ArchT-EGFP (ArchT) or a CAG-EGFP control virus. Three weeks later, optical fibers were bilaterally implanted above the injection site. One week later, rats were conditioned using a multi-trial contextual fear conditioning paradigm (five shocks spaced three minutes apart) on day one and tested for freezing to the context-alone on days two and three. The mPFC was only silenced on day one during contextual fear acquisition. Optogenetic silencing of mPFC cells during acquisition did not affect pre-conditioned freezing to the context or the rate of acquisition. However, ArchT rats showed enhanced freezing relative to controls during the light-free context-alone retention test on day two. Preliminary data also found that the enhanced freezing persisted when rats were tested again in the same context on day three, suggesting extinction is disrupted in ArchT rats. Furthermore, ArchT and control rats did not differ during acquisition or retention when re-conditioned (without optogenetic silencing) in a novel context, indicating that laser inhibition did not cause permanent cellular damage. Interestingly, despite two days of non-reinforced exposure to the original training context, ArchT rats exhibited increased baseline freezing to the novel context relative to controls. These data suggest that (1) mPFC activity at the time of acquisition is important for the future extinction of contextual fear memories and (2) that mPFC activity at the time of acquisition may also be involved in promoting contextual specificity.

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Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Program#/Poster#: 452.07/III6

Topic: G.01. Appetitive and Aversive Learning

Support: The Roskamp Fundation

Title: The use of synthetic TMT as a psychological stressor in a rodent model of PTSD.

Authors: *B. C. MOUZON, M. ALGAMAL, J. OJO, F. CRAWFORD;
The Roskamp Inst., Sarasota, FL

Abstract: Background: Various preclinical models of post-traumatic stress disorder (PTSD) use psychological stressors such as cat odor or fox urine and feces to induce PTSD-like symptoms in

tested animals. 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) is a highly volatile, water insoluble molecule that contains sulfur, an element in carnivores' diet and component of red fox feces that showed to illicit fear responses in prey animals. In naïve mice and rats, TMT induces a number of defensive and fear behaviors including freezing, indicating it is an innate threat stimulus. In this study, TMT was used as stressor alone or in combination with social and physical stressors to examine TMT effect on neuroendocrine system.
Methods: 12-month-old C57BL/6J mice were restrained using decapicone tubes for the whole duration of exposure (30 min). TMT (SRQBio, Florida, USA) was diluted to 1:10 in water and then 50 ul were dispensed on a cotton cloth per mouse. Control mice received water with no restrain instead of TMT. Mice were sacrificed 30 min after the end of exposure period. Plasma was collected for evaluation of corticosteroid and catecholamine concentrations.
Discussion:This work has high translational potential for patients affected by both PTSD or innate fear/phobia. Investigation using TMT as a threatening stimulus for rodents is critical to study and understand the nature and timing of treatment strategies to treat PTSD.

Disclosures: **B.C. Mouzon:** None. **M. Algamal:** None. **J. Ojo:** None. **F. Crawford:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Program#/Poster#: 452.08/III7

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH MH093412

Brain and Behavior Research Foundation 19417

Title: Female rats acquire and recall a conditioned safety signal more rapidly than males.

Authors: *A. R. FOILB, J. BALS, M. SARLITTO, J. P. CHRISTIANSON;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Veridical detection of safe versus dangerous cues is critical to survival and aberrant fear discrimination is a symptom of posttraumatic stress disorder (PTSD). More females are diagnosed with PTSD than males and clinical evidence suggests that women with PTSD have difficulty inhibiting fear responses. However, preclinical research into sex differences in safety learning is quite limited. Here, intact male and normally cycling female rats received fear discrimination training where a danger signal conditioned stimulus (CS+) co-terminated with a mild footshock and a safety signal (CS-) indicated the absence of shock. 24 h after conditioning

rats were given a discrimination test in the conditioning context in which freezing, an index of fear, was assessed during randomized presentations of the context alone, the CS+, or CS-. Importantly, all rats learned to discriminate between CS+ and CS-, yet females displayed a more robust phenotype evidenced by less freezing to the CS- compared to males within both the conditioning session and the subsequent recall test. This pattern suggests sex differences exist within the neural circuits that encode and recall safety information, which is the focus of ongoing study.

Disclosures: **A.R. Foilb:** None. **J. Bals:** None. **M. Sarlitto:** None. **J.P. Christianson:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH093412

Behavior Research Foundation Grant No. 19417

Title: Inactivation of the obitofrontal cortex impairs fear discrimination

Authors: M. C. SARLITTO, A. R. FOILB, *J. P. CHRISTIANSON;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Survival depends on flexible behavioral adaptation to shifting environmental risks and opportunities. PTSD is typified by inappropriate responses to environmental risks and so delineating the mechanisms which permit acquisition, recall, and flexible use of aversive and safe associations is critical to unraveling the neural bases of trauma-related disorders. In the realm of desirable associations (such as stimulus-reward pairings), the orbital frontal cortex (OFC) is critical for integrating outcome expectancies with flexible behavioral responses. To test the role of the OFC in cognitive flexibility within an aversive learning domain, we developed a fear discrimination procedure in which adult male rats readily shift behavioral responses depending on the presentation of either a shock paired conditioned stimulus (CS+) or a safety cue (CS-). We then used either reversible pharmacologic inactivation with the $GABA_A$ receptor agonist muscimol or chemogenetic silencing with the kappa-opioid DREADD (CamKII-KORD) to disrupt OFC functioning during fear discrimination recall tests. Muscimol significantly diminished the ability of adult male Sprague Dawley rats to discriminate between safety and danger cues. However, chemogenetic silencing had no effect. These contradictory findings

suggest there may be a part played by the OFC in modulating fear in response to changing dangerous and safe environments, but further investigation is needed to identify the specific population of OFC neurons involved.

Disclosures: M.C. Sarlitto: None. **A.R. Foilb:** None. **J.P. Christianson:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: CHIR postdoctoral fellowship

NIH R01 MH101528-01

Title: Brain-wide patterns of fos expression during fear learning correlate with emotional state rather than specific sensory stimuli

Authors: *J. CHO[1], S. RENDALL[2], J. GRAY[3];
[1]Genet., [2]Harvard Med. Sch., Boston, MA; [3]Harvard Med. Sch., BOSTON, MA

Abstract: In the hippocampus, *Fos* mRNA induction during learning identifies neural ensembles that encode a specific physical location, revealing a memory trace. In sensory cortex and other brain regions, the extent to which *Fos*+ neurons induced during associative learning correspond to a specific sensory representation is unknown. Here we generate high-quality brain-wide maps of *Fos* expression during auditory fear conditioning and recall, revealing 10-fold increases in *Fos*+ cells in a majority of 140 brain regions analyzed in fear conditioned mice. Contrary to the expectation that *Fos*+ neurons reveal specific sensory representations during learning, the brain-wide pattern of *Fos* expression following footshock alone recapitulates the pattern of brain-wide *Fos* expression seen following fear learning. The brain-wide pattern of *Fos* expression following fear recall is highly similar to that following fear conditioning, with no specificity for auditory areas. Our data suggest that *Fos* expression in sensory cortex and many other brain areas marks neuronal ensembles that represent distinct emotional states rather than specific sensory associations.

Disclosures: J. Cho: None. **S. Rendall:** None. **J. Gray:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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UCLA Depression Grand Challenge Fellowship Fund: MSF

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1PO1NS058489: DH, CG

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Centre for NeuroSkills: DH

Joseph Drown Foundation

Title: Diffuse traumatic brain injury enhances fear learning and dynamically alters processing within the auditory fear circuit

Authors: *A. N. HOFFMAN[1,2,3], J. LAM[1], Y. CAI[2], D. A. HOVDA[2,3,4], C. C. GIZA[2,3,5], M. S. FANSELOW[1,6];
[1]Psychology, [2]Neurosurgery, Brain Injury Res. Ctr., [3]Steve Tisch BrainSPORT Program, [4]Med. and Mol. Pharmacol., [5]Mattel Children's Hosp., [6]Psychiatry and Behavioral Sci., UCLA, Los Angeles, CA

Abstract: Traumatic brain injury (TBI) is labeled the signature injury of troops in recent combat operations, a population often exposed to stressful stimuli and emotional trauma. While TBI is typically known to impair learning and memory for neutral events, traumatic fear memories are enhanced after TBI, consistent with increased prevalence of comorbid TBI and post-traumatic stress disorder (PTSD) and other affective conditions such as general anxiety and depression. Changes in sensitivity to sensory stimuli are common after TBI, and might influence the encoding of traumatic events. Our lab has shown enhanced contextual fear after lateral fluid percussion injury (LFPI) when fear conditioned after injury with white noise (WN) cues, but not low frequency tones, paired with footshocks. Altered sensitivity to noise cues paired with footshocks might underlie overall enhanced contextual fear after injury. Therefore, we hypothesized that LFPI enhances contextual fear to WN-signaled conditioning due to injury-induced altered auditory processing. To determine how WN alone affects behavior after LFPI, injured and sham adult male rats were pre-exposed to WN stimuli (75dB) prior to fear

conditioning and subsequently tested for contextual and cued fear. Interestingly, LFPI rats showed significantly elevated freezing to both the WN and context during the pre-exposure session, as well as to the context after cued conditioning. We then investigated whether WN alone after LFPI alters functional activity and astrocyte reactivity within the well-defined auditory fear circuit including the lateral amygdala (LA), auditory thalamus (MGN), and auditory cortex (A1) as well as the dorsal dentate gyrus (DG) of the hippocampus by using Arc immediate early gene and GFAP immunohistochemistry. Data showed that noise-exposed LFPI rats had significantly elevated Arc induction in the ipsilateral LA, which is known as the sensory interface of the amygdala in fear conditioning. In contrast to the LA, Arc expression was reduced in ipsilateral A1 and DG in LFPI groups. Furthermore, GFAP expression was significantly greater in LFPI groups in the LA, A1, and MGN, with the greatest increases ipsilateral to the injury. These data provide implications for altered sensory processing after TBI, where otherwise neutral stimuli may adopt aversive properties and impact encoding of traumatic memories and contribute to adverse effects on psychological health and affective comorbidities.

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Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIMH RO1-MH62122

NIMH 5 F31-MH108257-02

Title: Lesions of the ventromedial prefrontal cortex reduce stress enhanced fear learning in a stimulus specific manner

Authors: *Z. T. PENNINGTON[1], A. S. ANDERSON[1], M. S. FANSELOW[2];
[1]Psychology, [2]Psychology and Psychiatry, UCLA, Los Angeles, CA

Abstract: Post-traumatic stress disorder is associated with both sensitization of stress systems and reductions in the volume and activity of the ventromedial prefrontal cortex (vmPFC). In order to address the causal contribution of the vmPFC to the sensitization of stress systems following traumatic stress, we assessed the impact of pre-training vmPFC lesions on two variations of a model designed to examine how acute traumatic episodes enhance subsequent

reactivity and learning about stressors. In Experiment 1, animals were given either 0 or 15 shocks during a 90-minute session in a distinct environment (Context A). After fear of Context A was assessed, all animals were given a single auditory startle stimulus in a novel environment (Context B), and fear of Context B was then examined. Animals shocked in Context A showed enhanced freezing in Context B following startle exposure relative to animals that had not been shocked, evidence of enhanced fear learning. Lesioned animals did not differ from controls with respect to shock reactivity or freezing in Context A. Additionally, they did not differ in their startle responses. However, lesioned animals that had received shock showed reduced fear relative to controls in Context B following startle exposure. This data is indicative of a counterintuitive reduction in stress sensitization by lesions of the vmPFC. As our laboratory has previously shown that vmPFC lesions can reduce contextual conditioning, Experiment 2 was carried out to assess the stimulus specificity of the observed effect. Animals were again given 0 or 15 shocks during a single 90-minute session (Context A). Subsequently, they received a single tone-shock pairing in a novel context (Context B), and fear of the tone-shock context, as well as the tone itself, was assessed. Here, although animals initially receiving 15 shocks froze more to both the tone-shock context and the tone alone, lesioned animals did not differ from controls. These data suggest that lesions of the vmPFC may selectively contribute to enhanced learning about contextual stimuli paired with an aversive event following acute traumatic stress.

Disclosures: Z.T. Pennington: None. **A.S. Anderson:** None. **M.S. Fanselow:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Program#/Poster#: 452.13/III12

Topic: G.01. Appetitive and Aversive Learning

Support: Wellcome Trust/DBT India Alliance Early Career Fellowship

Title: Genetically encoding an *In vivo* tag of synaptic plasticity associated with memory

Authors: *D. B. WEATHERILL[1], R. TANNA[2], K. C. MARTIN[3], S. CHATTARJI[4], M. S. FANSELOW[1];
[1]Psychology, [2]Ecology & Evolutionary Biol., [3]Biol. Chem., UCLA, Los Angeles, CA;
[4]Neurobio., Natl. Ctr. For Biol. Sci., Bangalore, India

Abstract: Due to the complexity of the mammalian brain, it has proven challenging to identify key sites of synaptic plasticity associated with memory in mammals. An approach to doing so would allow these sites to be probed for a causal role in memory recall. In recent years, several

groups have developed genetically-encoded methods for identifying neurons in rodents whose activity is involved in memory recall. However, such methods still leave open the question: at what site(s) within these neurons forming the neural circuit representing a memory is the memory actually stored? To address this question, we have developed a genetically-encoded method for selectively stabilizing an *in vivo* TimeSTAMP tag during a specified time window only at rodent synapses that have undergone synaptic plasticity. The TimeSTAMP tag consists of the fluorescent protein, mVenus, with a Hepatitis C Virus (HCV) NS3 protease flanked by two NS3-protease-specific cleavage sites fused between two structural domains of the fluorescent protein. We have shown that treatment with the HCV-NS3-specific protease inhibitor, asunaprevir, inhibits cleavage of the TimeSTAMP tag when fused to the synaptically-localized protein CaMKIIα. Inhibition of this cleavage allows the tag on newly synthesized TimeSTAMP fusion proteins to fold into a functional fluorescent protein. Moreover, in human embryonic kidney 293T cells, asunaprevir-induced stabilization/fluorescence of the TimeSTAMP tag can be completely blocked through co-expression of a fusion protein consisting of a drug-insensitive form of the HCV NS3 protease fused to the synapto-nuclear messenger protein, CRTC1. In neurons, as CRTC1 translocates away from activated synapses to the nucleus, fluorescent TimeSTAMP-tagged CaMKIIα should be restricted not only to the time window of asunaprevir treatment but also to synapses that have undergone synaptic plasticity. Following validation of this method in cultured neurons, it will be used *in vivo* to examine how auditory fear conditioning memory traces differ following weak versus strong training protocols.

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Poster

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH R03MH093781-03 (AMP)

SUNY Albany start-up funds (AMP)

Title: Incubation of Pavlovian fear responding: behavioral and neural correlates of recent and remote memory using between- and within-subject designs

Authors: *N. ODYNOCKI, P. R. ZAMBETTI, A. M. POULOS;
Psychology, Univ. At Albany State Univ. of New York, Albany, NY

Abstract: Pavlovian contextual fear conditioning in mice has often been used to model the neuro-behavioral basis of post-traumatic stress disorder. We have previously shown in rats that under specific conditioning parameters contextual fear responding is elevated between groups tested at remote versus recent retention intervals. This heightened fear at more remote retention intervals has been attributed to the notion that aversive memories can "incubate" across time [1, 2, 3]. Given this, very little is known about whether individual rodent subjects directly exhibit fear incubation across repeated testing. In our current work utilizing adult male C57BL/6J mice, we have replicated prior between-subject findings and added a within-subjects design as an alternative relevant model for PTSD and have begun exploring its neural basis. In the within-subjects design, mice were tested at both 3 and 28 days following conditioning time to assess context fear expression during remote retention interval after having been tested at a recent retention interval. Results initially revealed that within-subjects maintained a constant level of fear across time, contrasting with the incubation observed in the between-subjects design. Upon further investigation, individual differences in fear expression were apparent as some mice displayed increased levels of fear expression, while others exhibited reduced or stable fear expression across time. This finding complements the variable development of PTSD observed in humans. Furthermore, we examined the expression of immediate early gene product, c-Fos protein and the glutamate decarboxylase 2 gene product GAD65 protein in brain areas associated with contextual fear memory such as the basolateral amygdalar nucleus anterior part [4], lateral amygdalar, dorsal hippocampus, and prefrontal cortical areas. These analyses reveal differential levels of activation across groups. [1] Diven K. Certain determinants in the conditioning of anxiety reactions. *J Psychol Interdisciplinary and Applied.* 1937; 3: 291-308. [2] Eysenck HJ. A theory of the incubation of Anxiety/Fear Responses. *Behav Res and Therapy.* 1968; 6: 309-321. [3] Pickens CL, Golden SA, Nair SG. Incubation of fear. *Curr Protoc Neurosci*. 2013; 6:27. Unit 6. [4] Dong HW. *Allen Reference Atlas: A Digital Color Brain Atlas of the C57B1/6J Male Mouse.* 2008; John Wiley & Sons.

Disclosures: N. Odynocki: None. **P.R. Zambetti:** None. **A.M. Poulos:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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SUNY Albany start-up funds (AMP)

NIH GM109817 (AMK)

UTEP ORSP Grand Challenges Grant (AMK)

HHMI Persist (AMK)

Title: Neural tract tracing-based modeling of contextual fear circuits across development in rats

Authors: *A. J. SANTARELLI[1], K. N. NEGISHI[2], A. M. KHAN[2], A. M. POULOS[1];
[1]The State Univ. of New York At Albany, Albany, NY; [2]Univ. of Texas at El Paso, El Paso, TX

Abstract: Throughout development, mammalian species interact with their environment in ways that best facilitate survival. In adult mammals, both learning and responding to environmental threat are dependent upon the neural activity of the *basolateral amygdalar nucleus* (nomenclature: BLA; Swanson LW;[1]) and its corresponding afferents and efferents. We have recently identified that behavioral measures of contextual fear responding and the underlying BLA-based neural circuits differ at key development periods in the rat. Given these developmental changes, we predict that fear expression recruits the activation of distinct cortical pathways across development. To test this prediction, we infused a retrograde tracer into the BLA of male rats at postnatal day (PND) 19, 24, 35 and 90. The subjects were then exposed to a single-trial fear conditioning procedure and fear retrieval was assessed one day later. The immediate early gene product, c-Fos protein, was immunocytochemically detected and quantified throughout regions within the contextual fear circuit linked with BLA-projecting afferents. These functional connectivity data were analyzed using both principal components and stepwise regression techniques to determine the variability in freezing and BLA activity accounted for by each upstream region within the circuit. We then constrained the analysis based upon afferent activity to develop a model of fear retrieval that is consistent with the literature in adults but is novel among animals throughout PND 19, 24, and 35. We tested our model using a discriminant function analysis to predict age group membership based upon assigned weights of regions in the circuit. The analysis revealed that during the developmental emergence of fear retrieval the *lateral entorhinal area* provides the main cortical control for freezing; however, in older animals, this control shifts to the *prelimbic area*. Taken together, these data reveal age-dependent differences in the circuit regulating contextual fear retrieval. [1] Swanson LW. *Brain Maps: Structure of the Rat Brain*, 3rd ed. 2004; Elsevier

Disclosures: **A.J. Santarelli:** None. **K.N. Negishi:** None. **A.M. Khan:** None. **A.M. Poulos:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH R03MH093781-03 (AMP)

SUNY Albany start-up funds (AMP)

Title: Mapping time - dependent contextual processing and immediate early gene expression in hippocampal and extra - hippocampal regions in C57Bl/6J mice

Authors: *L. M. COLON, T. WINSTON, A. POULOS;
Psychology- Behavior Neurosci., Univ. At Albany State Univ. of New York, Albany, NY

Abstract: Contextual learning of fear requires a sufficient opportunity to encode the surrounding environment prior to the occurrence of an aversive footshock. It has been previously shown that this relationship between the lengths of time spent in the context prior to shock mediates level of context fear learning [1]. Numerous studies in both rat and mouse have demonstrated a vital role of the dorsal hippocampus in contextual learning [2, 3]. We sought to further examine this relationship between neural activity of hippocampal and extra-hippocampal circuits and the ability to successfully encode the conditioning context. In this study we mapped the expression of immediate early genes throughout dorsal and ventral hippocampus [4] as well as the basolateral amygdalar anterior and posterior part and the lateral entorhinal area [5] under varying placement-to-shock intervals in C57Bl/6J mice. Our preliminary results indicate increased patterns of immediate early gene expression throughout hippocampal and extra- hippocampal regions correspond with increased context exposure prior to footshock delivery.
[1] Wiltgen, B. J. et al., (2001). *Behavior neurosci 115*(1), 26.
[2] Kim, J. J., & Fanselow, M. S. (1992). *Science*, *256*(5057), 675-677.
[3] Chen, C. et al., (1996). *Behavior neurosci*, *110*(5), 1177.
[4] Dong HW. (2009). Proc Natl Acad Sci USA. 106(28): 11794-9.
[5] Dong HW. (2008) John Wiley & Sons.

Disclosures: **L.M. Colon:** None. **T. Winston:** None. **A. Poulos:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH8626450

Title: Hemodynamic changes in frontoparietal networks predict electrocortical population activity in visual cortex during aversive conditioning

Authors: *N. M. PETRO[1], L. F. GRUSS[2], S. YIN[3], H. HUANG[3], V. MISKOVIC[4], M. DING[3], A. KEIL[2];
[2]Psychology, [3]Biomed. Engin., [1]Univ. of Florida, Gainesville, FL; [4]Psychology, State Univ. of New York at Binghamton, Binghamton, NY

Abstract: Visual stimuli predicting threat facilitate perceptual biases and visual cortical responses are amplified relative to neutral stimuli. Selective visuocortical amplification of threat cues accompanying these perceptual biases has been demonstrated in multiple previous studies, but the mechanisms mediating these changes are not known. The current project aimed to identify large-scale neural networks functionally related to modulating visuocortical activity during aversive conditioning. Functional MRI, EEG, and electrocardiography were recorded simultaneously during a classical conditioning paradigm in which the orientation of grating stimuli (i.e. the conditioned stimulus, CS) predicted the presence (CS+) or absence (CS-) of a cutaneous electric shock (i.e. the unconditioned stimulus, US). Phase reversal of the gratings elicited a steady-state visual evoked potential (ssVEP) measured by the EEG, which is a strong measure of stimulus evoked visuocortical activity highly resilient to noise. Single-trial estimates of the ssVEP were used to build predictive models for the concurrently recorded whole-brain blood-oxygen-level dependent (BOLD) activity. CS+ relative to CS- trials during US pairing were associated with increased ssVEP amplitude, greater heart rate deceleration, and greater BOLD activation in primary visual, anterior insular, and temporal cortices bilaterally. Modeling BOLD activity using the single-trial ssVEP indices demonstrated specific BOLD-ssVEP coupling in primary and extended visual cortex over all trials, and CS+ relative to CS- specific activation in the calcarine, left inferior parietal, and bilateral dorsolateral prefrontal cortices. These results suggest that anterior extra-visual cortical areas are functionally related to amplified visuocortical responses during aversive conditioning. Future analyses may investigate the functional relation between these structures, including whether their conditional amplification occurs in a specific temporal sequence.

Disclosures: **N.M. Petro:** None. **L.F. Gruss:** None. **S. Yin:** None. **H. Huang:** None. **V. Miskovic:** None. **M. Ding:** None. **A. Keil:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Program#/Poster#: 452.18/III17

Topic: G.01. Appetitive and Aversive Learning

Support: Swedish Research Council

Title: Fear conditioning of social stimuli at proximal and distant locations in an immersive virtual reality environment

Authors: *G. KASTRATI, J. ROSÉN, S. HULTBERG, F. AHS;
Uppsala Univ., Uppsala, Sweden

Abstract: It is well established that intrusion of the near space surrounding the body by others increases autonomic activity and defensive behaviors. The role that spatial proximity plays in fear memory formation and extinction is however not well understood. We here tested whether fear conditioning of virtual characters displayed at near location in an immersive virtual reality environment was enhanced relative to fear conditioning of geometrical objects of different colors. One character or object was always displayed at a proximal egocentric location while being followed by the delivery of a mild electric shock (Proximal CS+), whereas another character or object was conditioned at a distant egocentric location (Distant CS+). Two distance matched control stimuli were never paired with shock (Proximal CS-, Distant CS-). Skin conductance responses (SCRs) served as conditioning index. Results showed that proximal characters elicited greater SCRs than proximal objects during a habituation phase prior to conditioning. During fear conditioning, CS differentiation was lesser to proximal relative to distant CSs due to enhanced responding to the Proximal CS-. However, fear acquisition was similar to social and inanimate CSs. During extinction, SCRs were consistently greater to proximal than to distant CSs, but the SCR difference between CS+ and CS did not differ as a function of location. These results suggest that safety learning is slowed within personal space. A reason for this might be enhanced generalization of fear to threats near the body, irrespective of whether the stimuli are social or non-social.

Disclosures: **G. Kastrati:** None. **J. Rosén:** None. **S. Hultberg:** None. **F. Ahs:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

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Program#/Poster#: 452.19/III18

Topic: G.01. Appetitive and Aversive Learning

Title: Remote but not recent pre-exposure increases generalization of fear learning in humans.

Authors: *B. D. YETTON[1], D. J. CAI[3], S. C. MEDNICK[2];
[1]Psychology, [2]Univ. Of California, Riverside, Riverside, CA; [3]Neurosci., Univ. Of California, Los Angeles, Los Angeles, CA

Abstract: Fear conditioning to a specific stimuli is stronger when an animal has been pre-exposed to the stimuli compared to when it is novel (Lubow, 1989). This pre-exposure may increase fear conditioning by forming a more robust representation of the stimuli to which fear can be associated (Rudy & O'Reilly, 1999). However, the timing of the pre-exposure moderates these effects (Kiernan & Westbrook, 1993). Rodents pre-exposed to a context either 1 day or 36 days before fear conditioning lead to greater fear response at test, and generalized to freezing in a novel, non-shocked context with longer delays (Wiltgen & Silva, 2007). Here, in humans, we investigate the effects of pre-exposure on the generalization of cued (as opposed to contextual) associated fear conditioning. 27 undergraduates (15 female, 18 years mean age) viewed 60 images from two categories (possible categories were animals, plants or tools) for 4 seconds (CS). Electric shock (US) was paired with one 80% of the images from one category at image offset, the other category received no shock (CS-). Subjects were pre-exposed for 15 minutes to CS+ stimuli either 1 or 8 days earlier. We hypothesized greater generalization of fear in the 8 day delay such that examples from the non-shocked, CS- category would elicit a stronger fear response. Using a composite of 2 convergent fear response quantification techniques (sound-evoked blink response and image-evoked skin conductance response), we find greater fear response to the non-shocked CS- items in the 8 day delay compared to the 1 day delay (independent samples t-test, $t=2.58$, $df=27$, $p<.05$). Additionally, fear levels are more robust (less variable) in the 1 day condition (1 day SD=0.32, 8 day SD=0.56), hinting at individual differences in the effects of pre-exposure at longer delays. Our results are of clinically relevance when considering the role of fear generalization in anxiety disorders (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015). A further non-pre-exposed control condition must be run to quantify the magnitude of the pre-exposure effect on fear conditioning.

Disclosures: B.D. Yetton: None. **D.J. Cai:** None. **S.C. Mednick:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 2R5GM06566-13

Title: Role of the Neuropeptide Y Y1 receptor antagonist, BIBP 3226 on NPY induced resilience in socially defeated Syrian hamsters

Authors: *K. KENNIEL[1,3], T. LACEY[2], R. KINGSTON[4], C. M. MARKHAM[3];
[2]Biol., [1]Spelman Col., Atlanta, GA; [3]Psychology, [4]Biol., Morehouse Col., Atlanta, GA

Abstract: Our lab utilizes an ethologically relevant model of social stress whereby defeated Syrian hamsters exhibit long lasting changes in behaviors, including increased submissiveness and a complete lack of territorial aggression, even when paired with a smaller, non-aggressive intruder (NAI). A small subset of hamsters, however, appears to be resilient to the effects of social defeat stress. Recent studies have suggested that neuropeptide Y (NPY), a widely distributed, 36-amino acid peptide, may function to reduce the effects of traumatic stress in a variety of animal models. We previously demonstrated that intracerebroventricular (icv) infusion of NPY induced a state of resilience in socially defeated hamsters by reducing submissive behaviors. The goal of the present study was to determine whether this effect is mediated by the NPY Y1 receptor. Hamsters were unilaterlly implanted with guide cannula aimed at the lateral ventricles. Following recovery, they were pretreated with the NPY Y1 receptor antagonist BIBP 3226 followed by an infusion of NPY. Results show that pretreatment with BIBP 3226 reversed the resilience inducing effects of NPY as indicated by a re-ermergence of submissive behaviors. These results provide support for the hypothesis that the resilience inducing effects of NPY in socially defeated hamsters is mediated, at least in part, by the NPY Y1 receptor.

Disclosures: **K. Kenniel:** None. **T. Lacey:** None. **R. Kingston:** None. **C.M. Markham:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 2R5GM06566-13

Title: The effects of exercise on resilience to social defeat stress in syrian hamsters

Authors: *C. M. MARKHAM[1,3], R. KINGSTON[2], J. BEST[3], M. EDWARDS[1];
[1]Psychology, [2]Biol., Morehouse Col., Atlanta, GA; [3]Biol., Spelman Col., Atlanta, GA

Abstract: Our lab utilizes an ethologically relevant model of social stress whereby defeated Syrian hamsters exhibit long lasting changes in behaviors, including increased submissiveness and a complete lack of territorial aggression, even when paired with a smaller, non-aggressive intruder (NAI). A small subset of hamsters, however, appears to be resilient to the effects of social defeat stress. Recent studies have suggested that voluntary exercise may promote resilience to a variety of stressors, including electric shock and chronic variable stress. However, no studies to date have examined the role of ethologically relevant stress on resilience. The aim of the present study was to determine whether voluntary exercise in the form of free access to a running wheel can inhibit the effects of social defeat in Syrian hamsters. Socially defeated hamsters were divided into the exercise (EX) or no-exercise (NEX) groups. Two weeks later, they were socially defeated and tested with a NAI. Our data indicate that hamsters in the EX group exhibited significantly lower levels of submissive behaviors compared to the NEX group, indicating that voluntary exercise promoted resilience to social defeat stress.

Disclosures: C.M. Markham: None. **R. Kingston:** None. **J. Best:** None. **M. Edwards:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH grant NS58867

NIH grant MH095948

Title: Fear conditioning increases GABA release from cerebellar stellate cells

Authors: *C. DUBOIS, S.-Q. LIU;
Dept. of Cell Biol. and Anat., LSUHSC, New Orleans, LA

Abstract: Post-Traumatic Stress Disorder (PTSD) is one of the most common psychiatric disorders and is associated with an increased risk of major depression, panic disorder and generalized anxiety disorder. One of the characteristics of PTSD is generalization of conditioned fear. It is thus important to understand the synaptic mechanisms underlying associative fear learning. The cerebellum is critical for fear memory consolidation and fear conditioning increases GABAergic transmission onto Purkinje cells, the sole output of the cerebellar cortex. Because inhibitory interneurons in the cerebellar molecular layer also innervate each other, we hypothesized that fear conditioning enhances inhibitory neurotransmission between interneurons and changes the activity of the interneuronal network. This is expected to alter the balance between excitatory and inhibitory inputs to Purkinje cells and thus information processing in the cerebellar cortex.

The conditioning paradigm consisted of 8 pairings of a 10 s tone (CS) which co-terminated with a 1 s foot-shock (US). Control groups included naïve mice never exposed to the CS or the US, and mice exposed to the US and CS 30 min apart (unpaired group). GABA release was evaluated by recording inhibitory postsynaptic currents (IPSCs) in stellate cells 15 h after the conditioning paradigm.

We found that the amplitude of evoked IPSCs (eIPSCs) was increased after fear learning indicating an enhanced inhibitory transmission. The paired pulse ratio of eIPSCs was decreased, which indicated a presynaptic effect. Spontaneous IPSCs frequency was consistently increased after fear conditioning without any change in amplitude. Furthermore, TTX-insensitive IPSC frequency, but not amplitude, was increased after conditioning suggesting that GABA release from stellate cells was potentiated by fear learning. Finally, the paired pulse ratio and amplitude of eIPSCs was not modified by the unpaired protocol. Spontaneous and miniature IPSCs frequencies were also not different from naïve animals. Together these results indicate that the stress induced by the conditioning was not responsible for the increase in GABA release from stellate cells.

We conclude that GABA release from cerebellar stellate cells is increased after associative fear conditioning but not stress alone. This suggests an important role of inhibitory neurotransmission in the consolidation of associative fear memory.

Disclosures: **C. Dubois:** None. **S. Liu:** None.

Poster

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Topic: G.01. Appetitive and Aversive Learning

Support: Whitehall Foundation 2014-08-67

RRG-Marquette University

Title: Sex-specific modulation of trace fear acquisition by pituitary adenylate cyclase activating-polypeptide signaling in the medial prefrontal cortex.

Authors: *A. J. KIRRY, M. R. HERBST, S. E. POIRIER, R. C. TWINING, M. R. GILMARTIN;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Women are more than twice as likely as men to develop post-traumatic stress disorder (PTSD), yet the neurobiological basis of this sex difference is unknown. Recently, pituitary adenylate cyclase activating-polypeptide (PACAP) signaling has been implicated in PTSD in females: a single nucleotide polymorphism in the gene encoding the PACAP type-1 receptor (PAC1R) is associated with PTSD symptom severity in women but not men (Ressler et al., 2011). PACAP is a highly conserved peptide important for mediating adaptive stress responses, and dysregulation of PACAP may contribute to maladaptive stress in PTSD. Additionally, aberrant PACAP signaling may contribute to PTSD through modulation of emotional learning. The PAC1R genetic polymorphism is associated with increased reactivity of fear circuitry to threat-related cues and impaired discrimination of threat and safety cues (Ressler et al., 2011, Stevens et al., 2014). Very little is known about how PACAP signaling normally participates in learning and memory, and previous work has focused primarily on male subjects. Here we examined the contribution of PACAP signaling in the prefrontal cortex of female and male rats to the acquisition of trace fear conditioning, an associative learning paradigm that requires the prefrontal cortex, hippocampus, and amygdala - memory structures which are also implicated in PTSD. Given that the medial prefrontal cortex (mPFC) mediates the effects of acute stress on learning selectively in females (Maeng et al., 2013), PACAP signaling in this region may be particularly relevant for fear learning in females. Adult Long-Evans rats were implanted with bilateral cannulas in the prelimbic area of the mPFC. Rats received injections of the PAC1R antagonist PACAP 6-38 (1, 2, or 3 mM, 0.5uL) into the prelimbic mPFC prior to trace conditioning in which a white noise conditional stimulus (CS) was paired with a foot shock unconditional stimulus (UCS) delivered after a 20-sec stimulus free trace interval. Rats were tested drug-free the following day to assess learned fear to the CS and the training context. We found that blocking PAC1R signaling in the prelimbic mPFC during training impaired the

formation of memory to the CS, but not context, in females, but not males, and the data suggest the influence of individual differences in the modulation of learning by PACAP. Prelimbic PACAP 6-38 did not impair performance in a delayed alternation working memory task, suggesting that prefrontal PACAP may selectively modulate prefrontal-dependent emotional learning. These results provide support for a sex-specific role of PACAP signaling within the prefrontal cortex in emotional memory formation.

Disclosures: A.J. Kirry: None. **M.R. Herbst:** None. **S.E. Poirier:** None. **R.C. Twining:** None. **M.R. Gilmartin:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Program#/Poster#: 452.24/III23

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R01MH097320

Title: Neural dynamics of fear conditioning

Authors: *S. YIN[1], Y. LIU[3], A. KEIL[2], M. DING[1];
[1]Biomed. Engin., [2]Dept. of Psychology, Univ. of Florida, Gainesville, FL; [3]Univ. of California, Davis, Davis, CA

Abstract: Work in the rodent models of fear conditioning has demonstrated the importance of the amygdaloid complex in mediating defensive responses to conditioned threat cues. In human imaging studies of aversive conditioning, however, activation of the amygdala is often not observed. In addition, roles of such brain structures as dACC and insula in fear learning remain not well-understood. We recorded simultaneous EEG-fMRI data from 18 subjects during a classical differential fear conditioning paradigm. Two Gabor patches (45° and 135°) were used as conditioned stimuli (CSs). One Gabor patch, the CS+, was occasionally paired with an aversive human scream (US; 25% reinforcement rate), whereas the other Gabor patch, the CS-, was never paired with the US. Single-trial BOLD responses to CS+unpaired and CS- were estimated using the beta-series method in three ROIs: amygdala, insula, and dACC. Representational dissimilarity matrix (RDM) was computed for each ROI using a moving window approach. We report the following results. First, activities in the three ROIs significantly declined over the course of the conditioning session, suggesting habituation. Second, the extent of activity decline in the amygdala, but not in the dACC and insula, predicted behavioral changes assessed by heart rate. Third, concomitant with the BOLD activity decline, representational

dissimilarity also declined significantly, suggesting reduced distinctiveness of the two cues in their evoked multivoxel patterns. We further analyze and report changes in EEG patterns and correlate these changes to the concurrently recorded BOLD to assess their anatomical underpinning.

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Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Topic: G.01. Appetitive and Aversive Learning

Support: Howard Hughes Medical Institute

Helen Hay Whitney Foundation

Title: Virtual burrow assay for measuring aversion to conditioned stimuli

Authors: ***C. E. SCHOONOVER**, A. J. P. FINK, R. AXEL;
Neurosci., Columbia Univ., New York, NY

Abstract: Traditional assays of aversive classical conditioning often rely upon measures of behavioral phenomena that play out over many seconds, such as time spent freezing or avoidance of a conditioned stimulus. These quantities are difficult to relate to the neuronal events that govern them, which unfold on a timescale of milliseconds. We have developed an assay with the aim of detecting behavioral transitions at a temporal resolution that permits comparison with neuronal processing. The Virtual Burrow Assay (VBA) is designed to simulate the naturalistic scenario in which a mouse at the threshold of its burrow continuously evaluates whether or not to retreat to safety. It consists of a light enclosure (virtual burrow) affixed to a frictionless rail that a head-fixed mouse can displace along its body's anterior-posterior axis. A laser displacement sensor tracks the position of the burrow, yielding a continuous one-dimensional variable. This allows determination of when an animal retreats in response to a given stimulus, pulling the enclosure around its body ("ingress"). Following classical conditioning, in which a CS+ odorant is paired with foot-shock and a CS- odorant is not, mice ingress in response to the CS+ alone, a behavior that extinguishes after repeated presentation. The VBA thus affords single-trial determination of whether a given stimulus evokes an aversive response and resolves the precise timing of ingress, permitting examination of the neuronal state that precedes this behavioral transition. The assay likely accommodates modalities other than olfaction and is compatible with

standard electrophysiological and optical methods for measuring and perturbing neuronal activity.

Disclosures: **C.E. Schoonover:** A. Employment/Salary (full or part-time): Columbia University. **A.J.P. Fink:** None. **R. Axel:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 452.26/III25

Topic: G.01. Appetitive and Aversive Learning

Title: Parametric characterization of a novel one way active avoidance learning on a treadmill using head fixed mice

Authors: ***H. JIE**[1], T. GEILLER[3], S. ROYER[3], J.-S. CHOI[2];
[1]Korea Univ., Seoul-City, Korea, Republic of; [2]Korea Univ., Seoul, Korea, Republic of; [3]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Avoidance is a key diagnostic criterion for fear-related disorders. Here we developed a one-way active avoidance (OWAA) learning using head-fixed mice on a treadmill (modified from Royer et al., 2012). In Exp1, effect of auditory vs. visual conditioned stimulus (CS) on OWAA learning was tested using either a visual (LED, 20 lux) or an auditory (1 kHz, 90 dB) stimulus that preceded the upcoming air puff (0.1 mPa, 4.6 L/m, via 3 mm nozzle) unconditioned stimulus (US). The US was avoidable by walking forward (criterion: 2 cm) or stopping (criterion: 5 s) to the CS, depending on the paradigm (Go or No-go, respectively). Animals ran on a treadmill 40 minutes a day for 1 to 3 days, and then were given 3 to 10 CS-only trials as a habituation. Training consisted of 30 trials per day, for two consecutive days. The CS lasted a maximum of 15 s, and the US was presented after 10 s of the CS onset time. The US lasted a maximum of 5 s. In each sensory modality, animals were divided into two training groups; Go or No-go. When the visual CS was used, the avoidance rate of No-go group (n=11) was significantly higher than that of Go group (n=11, $F[1,20] = 6.590$, $p < .05$). When the auditory CS was used, the avoidance rate of the No-go group (n=10) was also significantly higher than that of Go group (n=10, $F[1,18] = 5.694$, $p < .05$). In addition, when we analyzed rate of change of avoidance, the visual CS group was significantly higher than the auditory CS group ($F[1,19] = 4.812$, $p < .05$). To find out whether the location of visual stimulus could change the OWAA learning, we conducted Exp 2. We manipulated location of the visual CS along the midsagittal line of the mouse head, dividing it into two different angles while maintaining the distance equal; 20°, 70°. The experimental procedure was identical to Exp 1 except criterion (10 cm in Exp 2) to

make the task difficult. The avoidance rate was higher for the 70° group (n=5) than the 20° group (n=4, $F[1,7] = 11.866$, $p < .05$). Taken together, these results suggest that the visual stimulus could induce acquisition of OWAA learning easily than the auditory stimulus when the location was controlled precisely, unlike previous studies that visual stimulus was presented at ceiling of chamber. These results indicate that not only CS modality but also spatial location of stimulus is important to learn OWAA in mice.

Disclosures: **H. Jie:** None. **T. Geiller:** None. **S. Royer:** None. **J. Choi:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 452.27/III26

Topic: G.01. Appetitive and Aversive Learning

Title: Behavioral correlates of neuronal allocation in auditory fear conditioning

Authors: *J. L. STRAIGHT, C. MCINTYRE;
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Abstract: One proposed mechanism for linking related memories is their allocation to overlapping ensembles of neurons throughout the brain. Recent studies suggest that transient, experience-dependent increases in cellular excitability may provide a mechanism to link memories that are qualitatively and temporally connected. Rats exposed to a novel context one hour before weak inhibitory avoidance (IA) training develop robust long-term memories. This enhancement has been attributed to synaptic tagging and capture, by which plasticity-related proteins generated in response to the novel context help strengthen the weak IA memory. Interestingly, novel context exposure less than one hour before weak IA training does not promote long-term memory formation. These findings have been replicated using several learning tasks, but the temporal dynamics by which novelty affects neuronal allocation in aversive memory formation are not fully understood. We exposed rats to a novel context and tone 30min or 5min before auditory fear conditioning (AFC) to a different context and tone. One day later, rats were given retrieval tests for both experiences and freezing was measured. Here, we show that novel context and tone exposure 5min, but not 30min, prior to AFC enhances learning. This observation suggests that neuronal allocation may be altered in the 30min condition. To test this hypothesis, we will use *Arc* catFISH to examine the relationship between neuronal ensembles activated by both retrieval tests. Our results add to the behavioral tagging literature and suggest that neuronal allocation is dynamically regulated following exposure to novelty.

Disclosures: **J.L. Straight:** None. **C. McIntyre:** None.

Poster

452. Fear and Aversive Learning and Memory: Acquisition

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Program#/Poster#: 452.28/III27

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA Grant DA037216-02

VA Grant IBX000741B

Title: The role of acid sensing ion channel 1A in Pavlovian reward learning

Authors: ***A. GHOBBEH**[1], S. ALAM[1], R. J. TAUGHER[1], R. FAN[1], R. T. LALUMIERE[2], J. A. WEMMIE[1];
[1]Psychiatry, [2]Psychological and Brain Sci., The Univ. of Iowa, Iowa City, IA

Abstract: Acid Sensing Ion Channel 1A (ASIC1A) contributes to synaptic transmission and plasticity, is abundant in the amygdala, and is required for Pavlovian fear conditioning. ASIC1A is also abundant in the nucleus accumbens (NAc) and recent studies suggest that ASIC1A in the NAc plays a role in addiction-related behaviors to cocaine and morphine. Because the nucleus accumbens is known to be critical for other reward-related behaviors including Pavlovian conditioning to rewards, we hypothesized that ASIC1A might contribute to reward-related learning and memory in general. We therefore tested multiple non-drug rewards utilizing a Pavlovian conditioning paradigm to explore the possible involvement of ASIC1A in reward-related learning and memory. We found Pavlovian reward conditioning to be intact in *Asic1a*$^{-/-}$ mice, relative to *Asic1a*$^{+/+}$ controls. These data suggest that despite a role in Pavlovian fear conditioning and effects on addiction-related behaviors to cocaine and morphine, ASIC1A disruption has little or no effect on Pavlovian reward learning to non-drug rewards. Together these data suggest that the role of ASIC1A in reward-motivated behaviors may be specific to drugs of abuse.

Disclosures: **A. Ghobbeh:** None. **S. Alam:** None. **R.J. Taugher:** None. **R. Fan:** None. **R.T. LaLumiere:** None. **J.A. Wemmie:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 453.01/III28

Topic: G.02. Motivation

Title: Lateral habenular-projecting hypothalamic neurons regulate food preference in rats

Authors: *R. M. O'CONNOR, P. J. KENNY;
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Abstract: Rates of obesity are on the rise worldwide, resulting in a growing threat to public health. Pharmacotherapies that safely reduce body weight in obesity remain elusive, partially due to our incomplete knowledge of the complex neuronal mechanisms that control food choice (palatable high-calorie versus less palatable low-calorie food). The lateral hypothalamus (LH) is considered a critical node in the maintenance of energy homeostasis. The development of obesity in rats is associated with a deficit in LH sensitivity to rewarding stimuli and a switch in preference towards palatable calorically dense food items. The habenula is a distinct set of nuclei linking forebrain and midbrain structures and is divided into two principal parts termed the medial habenula (MHb) lateral habenula (LHb). The LHb has been described as a "preference center" that exerts negative influence over motivated behaviors by inhibiting midbrain dopamine neurons. A major input to LHb originates from LH, providing a mechanism by which metabolic status can be transmitted to habenular neurons involved in regulating motivated behavior and preference. Here, we tested the hypothesis that LH projections to LHb may regulate alterations in the motivational value of food and food preference that emerge during the development of obesity. To target the LHb-LH pathway in rats, we delivered a retrograde AAV2/5-Cre-eYFP virus into LHb and cre-inducible "excitatory" (hM3Dq) DREADD into LH, thereby placing the LH-LHb pathway under experimenter control. We found stimulation of this pathway increased the motivational value of standard food pellets in rats, as measured by increased willingness to respond for food rewards. Conversely, ablation of this pathway, accomplished using Cre-inducible diphtheria toxin (DTA), decreased the motivational value of standard food pellets. Interestingly, DREADD-mediated stimulation of the LH-LHb pathway decreased consumption of palatable energy-dense food, whereas ablation of this pathway increases consumption of the palatable food; opposite effects of these manipulations were observed when only standard (less palatable) chow was made available. These data suggest that the LH-LHb system plays an important role in regulating the motivational value of, and preference for, palatable food. As such, the LH-LHb pathway may represent an important target for therapeutic intervention in obesity.

Disclosures: R.M. O'Connor: None. **P.J. Kenny:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Topic: G.02. Motivation

Support: NIH Grant 2T32MH087004-06

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NIH Grant 5R01MH104559-02

Title: Lateral habenula orexin receptor-2 signaling controls aggression reward

Authors: *M. FLANIGAN[1], H. ALEYASIN[1], A. TAKAHASHI[1,2], E. S. CALIPARI[1], C. MENARD[1], M. PFAU[1], S. J. RUSSO[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Elevated interpersonal aggression and violence are common symptoms of multiple psychiatric disorders and represent a significant global health issue that lacks both sufficient therapeutic strategies and satisfactory understanding of relevant neuropathologies. Recent neuroimaging studies in humans suggest that aggression in psychiatric patients may result, in part, from the inappropriate activation of reward circuitry in social contexts. The neuropeptide orexin, which is produced exclusively in the lateral hypothalamus (LH), has been implicated in a broad array of motivational behaviors, including conditioned responses to rewarding or aversive stimuli like drugs of abuse and social stress. In this study, we use in-situ hybridization, immunohistochemistry, qPCR, in-vivo viral-mediated gene transfer, and in-vivo calcium imaging (fiber photometry) to investigate the role of orexin-containing projections to the lateral habenula (lHb) in aggression and a model of aggression reward we deem aggression conditioned-place preference (CPP). Our results indicate that orexin-positive neurons are activated during aggression, and orexin release from terminals in the lHb increases aggression and aggression CPP through indirect inhibition of the lHb. This appears to occur via activation of OxR2 on GAD65-positive neurons in the lHb. The results of this study illustrate the importance of orexin in the rewarding properties of aggression and provide a previously undefined role for orexin signaling in the lHb.

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Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Program#/Poster#: 453.03/III30

Topic: G.02. Motivation

Support: NIMH Grant 5R01MH093667-05

Title: Neuroanatomical and electrophysiological studies of Gad2-expressing neurons in the lateral habenula

Authors: *A. W. WALKER, L. A. QUINA, G. R. MORTON, Y.-W. A. HSU, A. WEI, E. E. TURNER;
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Abstract: Background/Objectives: The habenula is a paired nucleus in the epithalamus, comprised of medial and lateral subnuclei, both of which project to the midbrain and pontine tegmentum via the fasciculus retroflexus. Recently, we established that a subset of neurons in the lateral habenula (LHb) express Gad2, an enzyme of GABA synthesis, which distinguishes them from the medial habenula (MHb) and other adjacent brain nuclei. To date, the function of LHb output circuits is not well understood, as the only intensively studied target of the LHb is the rostromedial tegmental nucleus (RMTg). Therefore, the objectives of these studies are to characterize the anatomy and physiology of Gad2 neurons in the LHb and use Gad2 as a genetic tool to dissect and specifically manipulate pathways from the LHb. **Methods:** *In situ* hybridization and immunohistochemical techniques were used to identify expression of other markers in the LHb. To establish projection patterns from LHb Gad2 neurons, transgenic mice with an IRES-Cre expression cassette targeted to the Gad2 locus ($Gad2^{Cre}$ mice) were injected with a Cre-dependent adeno-associated virus (DIO-AAV-GFP) and efferent fibers were examined at all brain levels. Finally, $Gad2^{Cre}$ mice in combination with optogenetic and whole-cell patch clamp techniques were used to understand the physiology and function of LHb Gad2 output circuitry. **Results:** LHb Gad2 neurons do not contain Gad1 or Vgat (responsible for synaptic packaging of GABA) mRNA. Rather, expression of the vesicular glutamate transporter Vglut2 throughout the LHb suggests these neurons are glutamatergic. Electrophysiological studies will further elucidate the fast neurotransmitter(s) utilized by these neurons. Analysis of efferent fibers revealed that LHb Gad2 neurons contribute few if any projections to the RMTg-VTA pathway, but instead specifically innervate hindbrain regions including the dorsal raphe, median raphe, and adjacent nuclei. Genetic and electrophysiological approaches will be used to identify the neurochemical phenotype of postsynaptic neurons. **Conclusions:** These studies have identified a unique subset of neurons in the LHb that seem to synthesize GABA but have no obvious mechanism to package GABA for synaptic release. Instead, LHb expression of Vglut2

suggests these neurons may use glutamate as their primary neurotransmitter. Furthermore, virally-mediated tract-tracing of LHb Gad2 neurons revealed exclusive projections to the raphe, with minimal innervation of the RMTg. Therefore, $Gad2^{Cre}$ mice in combination with optogenetic strategies will allow for the dissection and manipulation of a novel, genetically defined LHb-hindbrain pathway.

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Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Topic: G.02. Motivation

Support: UNAM-PAPIIT IN216214

CONACYT CB-238744

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Title: Hypothalamic and midbrain peptidergic-aminergic pathways modulate intrinsic GABAergic signaling in the lateral habenula: a study using *In vivo* juxtacellular labelling, retrograde tracing, IHC and confocal microscopy

Authors: *L. ZHANG[1], V. S. HERNANDEZ[1], L. E. EIDEN[2];
[1]Physiology/Medicine, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [2]Sec Molec Neurosci,, NIMH-IRP, NIH, Bethesda, MD

Abstract: We have recently reported by in vivo juxtacellularly-labelled neuron morphology the presence of axons immunopositive for vGAT, and branching inside the LHb, particularly in the medio-central subnucleus of the lateral habenula (LHbMC) in rat. The role of these neurons in escape behavior during 'meta-stress coping' was also reported (Zhang et al, Frontiers Neural Circuit 2016). The presence of peptidergic/aminergic nerve terminals in this region, particularly to the GABAergic interneurons, is of great interest in understanding peptidergic/aminergic modulation of GABAergic transmission in the habenula. By using numerous antibodies and ISH probes, we found that several peptidergic pathways to habenula are highly sub-field specific. Vasopressin immunopositive fibers of hypothalamic origin are distributed in a highly selective manner in the medial subdivision of the LHb (LHbM). The fibers were grouped mainly in three subnuclei: the superior subnucleus (LHbMS), the central subnucleus (LHbMC), and the marginal

subnucleus (LHbMMg), and the highest density of VP+ fibers was observed inside the LHbMC. We also examined the expression of other relevant neurochemical markers of hypothalamic and midbrain origin: tyrosine hydroxylase (TH), dopamine beta hydroxylase (DBH), serotonin transporter (SerT), somatostatin (SOM), enkephalin (ENK), substance P (SP), vasoactive intestinal polipeptides (VIP), pituitary adenylate cyclase activating peptide (PACAP), calretinin (CR), calbindin (CB), as well as G protein-coupled inwardly-rectifying potassium channel 1 and 2 (GIRK1 and GIRK2). A strong overlap was observed within the LHbMC between VP and midbrain aminergic projections (immunoreactivity to TH and SerT, but not DBH). These results may indicate that: (1) the LHbMC is a key region modulated by subcortical aminergic pathway; and (2) VP innervation may contribute to the robustness of the modulatory mechanism for LHb function, which is also regulated by midbrain aminergic pathways. The region of strong immunostaining for SOM, CR, and CB was also similar to VP. The expressions of ENK, SP, and GIRK1 and GIRK2 were not similar to that of VP, particularly low in LHbMC. CB immunopositive somata were predominantly located in the LHbMC, and CB+ axons seemed to project to fasciculus retroflexus. These results suggest that the pathways involving in motivations and reward, such as the hypothalamic vasopressinergic and the midbrain catecholaminergic systems, may modulate the habenular intrinsic GABAergic signaling. Supported by grant: PAPIIT IN216214, CONACYT 238744

Disclosures: **L. Zhang:** None. **V.S. Hernandez:** None. **L.E. Eiden:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Topic: G.02. Motivation

Support: NSERC Grant GRPIN-2015-05018

Title: Stimulation-induced Fos-like immunoreactivity following electrolytic lesions of the dorsal diencephalic conduction system

Authors: ***M. FAKHOURY**, D. VOYER, D. LÉVESQUE, P.-P. ROMPRÉ;
Univ. of Montreal, Montreal, QC, Canada

Abstract: Intracranial self-stimulation (ICSS) holds a great promise in understanding the neural elements involved in reward and goal-directed behaviors, and offers a valuable tool for their anatomical mapping and functional characterization. We have previously shown that electrolytic lesions of the dorsal diencephalic conduction system (DDC) result in attenuations of the

rewarding effectiveness of ICSS within multiple brain regions, including the lateral hypothalamus (LH). The present work sought to extend these findings by describing the effect of a DDC lesion on the expression of the immediate early gene c-fos following LH stimulation. For this purpose, rats were implanted with monopolar electrodes and divided into three groups; the first two groups were trained to self-stimulate at the LH, whereas the third group received no stimulation and served as a control. Among the two groups that were trained for self-stimulation, one of them received a lesion at the DDC and was tested for ICSS on the subsequent 5 days. On the last day of testing, control rats were placed in operant chambers without receiving any stimulation, and the remaining rats were allowed to self-stimulate for 1h. All rats were then transcardially perfused and their brain collected for Fos-like immunoreactivity (FLIR). As previously shown, a lesion at the DDC resulted in significant attenuations of the rewarding effectiveness of LH stimulation. Results also show a higher FLIR in several reward-related areas following LH stimulation, especially in the hemisphere ipsilateral to the stimulation electrode. Compared to non-lesioned rats, animals that received a lesion had lower FLIR in certain brain regions, suggesting that those regions that were activated by the stimulation may be functionally interconnected with the DDC.

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Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Topic: G.02. Motivation

Support: 1R01MH101377-01

Title: A hypothalamic circuit controlling aggressive motivation and action

Authors: ***A. L. FALKNER**[1], R. TREMBLAY[1], I. SCHMITT[1], B. RUDY[1], M. HALASSA[1], D. LIN[2];
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Abstract: Social actions, like sex or aggression, may be preceded by a motivated internal state that promotes animals to seek out opportunities to perform these behaviors. While significant progress has been made indentifying neural substrates that are involved in social action, it has been more difficult to assess the neural mechanisms of these underlying motivated or seeking states. The hypothalamus, and in particular the ventromedial hypothalamus, ventrolateral area (VMHvl), now has an established role in intermale aggression. Stimulation of this area promotes

attack and neurons in this area are active during aggressive action. We have recently expanded the role of this area to be critical for flexible "proactive" aggression-seeking behavior. Using a social-operant task, where male mice can seek out brief and repeated attack opportunities, we find that single neurons in the VMHvl respond during this motivated seeking phase in addition to the social action phase, and changes in population activity recorded using fiber photometry track changes in task learning and extinction. Optogenetic stimulation of this area accelerates trial-to-trial response initiation latency, promoting changes in moment-to moment aggressive motivation. In addition, we find a new role for an anatomically segregated population of inhibitory neurons on the lateral edge of the VMHvl (the VMHvl "shell"). These neurons send strong direct inhibitory current to VMHvl neurons and population recording of these neurons shows that activity is decreased during aggressively motivated seeking behavior. Consistent with this, optogenetic inactivation of these GABAergic neurons is also sufficient to accelerate trial-to-trial aggression seeking behavior. Lastly, we used *in vivo* FRET photometry to detect changes in chloride concentration in the VMHvl during aggression seeking behavior and found that inhibition to this area is reduced during the motivated seeking phase. Together these data suggest that local hypothalamic inhibitory input to the VMHvl behaves as a permissive gate during aggression seeking behavior and the strength of this input is relaxed as animals prepare for and seek out future aggressive action.

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Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Topic: G.02. Motivation

Support: NIH Grant GM109817 to AMK

HHMI PERSIST Education Grant to AMK

Title: Elaboration of hypothalamic chemoarchitecture of the adult male rat: A high spatial resolution mapping study of melanin-concentrating hormone, hypocretin/orexin, and calbindin immunoreactivities in multiple subjects

Authors: ***C. D'ARCY**[1], A. MARTINEZ[2], L. F. ARANDA[2], H. F. L. CERVANTES[2], L. E. CHACON[2], R. P. CORDERO[2], V. FERNANDEZ[2], G. A. GARCIA[2], S. HOLGUIN[2], A. JAQUEZ[2], T. G. MIRAMONTES[2], B. MONTAÑO[2], P. C. MUÑOZ[2], I. R. VALENZUELA[2], J.

S. YU[2], A. M. KHAN[2];
[1]Biol. Sci., Univ. Texas El Paso, El Paso, TX; [2]Biol. Sciences, HHMI PERSIST Program: Brain Mapping & Connectomics Track, Univ. of Texas at El Paso, El Paso, TX

Abstract: The hypothalamus is an important brain structure for the integration of autonomic, neuroendocrine, and somatomotor function. The precise circuitry and neuronal populations underlying these functions; however, remain poorly resolved in terms of spatial organization. This study expands our previous report (SfN Chicago, 2015; poster #616.08) of the distribution and interactions of hypothalamic peptides and their mapped locations to the Swanson rat brain atlas (Brain Maps: Structure of the Rat Brain, 3rd ed., 2004).A cohort of freshmen undergraduates were recruited and taught formal atlas-mapping techniques within an HHMI-funded laboratory-based course: Brain Mapping & Connectomics. The distributions of hypocretin/orexin (H/O), melanin-concentrating hormone (MCH) and calbindin (CalB) were mapped from the brains of multiple animals in order to generate a canonical chemoarchitectural atlas of the adult male rat brain. H/O and MCH both play a role in feeding control and energy homeostasis, while calbindin helps buffer calcium ion concentration throughout the brain. Using the adjacent tissue series from animals used by the previous student cohort, we extended the chemoarchitectural atlas effort by staining for H/O, MCH, and CalB using triple-label immunofluorescence. The immunoreactivities for these peptides in the hypothalamus were imaged using wide-field high resolution microscopy and mapped to the Swanson atlas using the indexed Nissl-stained tissue series as a reference for cytoarchitectural analysis.Students completed comprehensive sets of maps that allowed for expression patterns of H/O, MCH and CalB to be compared across brains while assessing individual variability. When staining in the LHA, CalB and H/O were observed to co-localize in some neurons; however, not all H/O neurons are CalB-positive and not all CalB neurons are H/O-positive. Furthermore, MCH does not co-localize with either CalB or H/O. There were appositions suggesting putative synapses between CalB-positive neurons with both MCH and H/O fibers. In addition, H/O-ipositive fibers displayed a tendency to make appositions with MCH neurons. This work marks the continuation of chemoarchitectural mapping of rat hypothalamic peptides, their novel relationships, and the contribution of freshmen to map the brain as part of a teaching lab. The chemoarchitectural atlas under development here will aid in experimental design and data interpretation for investigators studying the functionality of the rat hypothalamus.

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Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Support: NIH Grant GM109817

UTEP ORSP Grand Challenges Grant

Title: Initial chemoarchitectural analysis of the infralimbic, prelimbic, and anterior cingulate areas of cerebral cortex in the adult male rat: Novel maps within a canonical atlas space

Authors: *K. NEGISHI[1], S. N. RODARTE[2], O. KOLENC[2], A. M. KHAN[2];
[1]UNIVERSITY OF TEXAS AT EL PASO, El Paso, TX; [2]Univ. of Texas at El Paso, El Paso, TX

Abstract: A cellular level structural account for "prefrontal" cortical functions is confounded, in part, by the sheer diversity of cortical neurons, which vary in their neurophysiological, connectional, spatial, morphological and neurochemical properties. These properties also vary across cortical regions and methodological constraints prevent their simultaneous examination. Here, we identify certain chemoarchitectural features of the rat *cingulate region* (CNG; Brodmann, 1909) using a Nissl-based approach, and map this chemoarchitecture in a canonical reference atlas space for the rat brain (Swanson, Brain Maps: Structure of the Rat Brain, 3rd ed; 2004).
Specifically, we performed immunohistochemical staining to label parvalbumin (P), somatostatin (S) and hypocretin/orexin (H/O) in coronal sections spanning Swanson atlas levels 8-11. Wide-field epifluorescence microscopy was used to visualize stained fibers and cell bodies within the anterior cingulate area (ACA), the prelimbic (PL) and the infralimbic area (ILA). Areal and laminar demarcations were assigned using an adjacent Nissl-stained series and the cytoarchitectonic criteria of the atlas. Precise mapping of cell bodies and fibers was achieved by overlaying the Nissl-derived boundaries on corresponding fluorescence images, which were, in turn, aligned by their blood vessels. We also used high-resolution confocal microscopy on the same sections to examine somatic morphology and putative synaptic interactions among the immunolabeled groups.
Preliminary staining of the CNG revealed differences across the dorsoventral axis. In particular, P and S expressing neurons were denser in the ACA and a dorsal part of the PL than the ventral PL and ILA. H/O fiber staining followed the opposite trend: the highest H/O axon density was found in the ILA while dorsally adjacent structures were gradually less dense. Preliminary confocal analysis in the CNG demonstrated that there are putative appositions of H/O-labeled axons with P but not S cell bodies, suggesting that H/O projection neurons target CNG subpopulations selectively.

Our canonical maps and spatial framework can be used as a platform to contextualize neuronal features and other mapped datasets, such as cannula placements and results from tract tracing studies.

Disclosures: **K. Negishi:** None. **S.N. Rodarte:** None. **O. Kolenc:** None. **A.M. Khan:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Support: NIH Grant GM109817 to AMK

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NIH NRSA Fellowship 1F31DK093331 to AMS

NIH Grant DK081538 to VHR

Title: Using multi-scale, mixed media methods to visualize and map electrophysiologically identified glucose-sensing neurons within canonical brain atlas space

Authors: *E. PERU[1], A. M. SANTIAGO[2], V. H. ROUTH[2], A. M. KHAN[3];
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Abstract: Although glucose-sensing (g-s) neurons in the brain have well-characterized biophysical properties that have made them objects of intensive investigation, little is known about their precise distributions within various brain regions. In particular, it remains unknown whether g-s neurons exhibiting a particular electrophysiological fingerprint (e.g., increased excitability in the presence of glucose) are associated with unique regionalization in the brain and/or display a unique morphological phenotype. Here, we have established a working protocol to identify and morphologically characterize dye-filled electrophysiologically identified g-s neurons within tissue slices of the mouse hypothalamus, and to map these labeled neurons using the cytoarchitecture within the tissue slice as a reference.
During patch-clamp recordings g-s neurons in brain slices were dialyzed with Lucifer yellow (LY) in the recording pipette. After recording, the tissue slices were fixed in 2% formaldehyde and stored unfrozen in a cryoprotectant at –20°C until processed further. Slices were rinsed in

buffered saline, immunostained with a fluorescently tagged anti-LY antibody, and counterstained with the fluorescent Nissl stain, Neurotrace. Confocal stacks of imaged neurons were imported into Volocity software (Perkin-Elmer) to visualize the native labeled morphology of the neuron, and into Neurolucida software (MicroBrightfield) to 3-D reconstruct the neuron. Tissue slices were then stained with the Nissl stain, thionin (skipping dehydration, clearing and rehydration steps to minimize disruption of the tissue slice integrity), and imaged as a z-series under bright field illumination using a wide-field microscope equipped with a motorized stage. Careful fiducial-based alignments of the fluorescent and bright field z-series allowed visualization of the labeled neuron, in fluorescence, in relation to the cytoarchitectonic boundaries delimited by the thionin stain, in bright field. The location of each neuron was plotted onto the appropriate digital atlas plate within a mouse brain atlas (Paxinos & Franklin, *The Mouse Brain in Stereotaxic Coordinates*). This method allowed us to map neurons to the ventrolateral subdivision of the ventromedial hypothalamic nucleus. In sum, we have used a multi-scale, mixed media (fluorescence, histochemical) approach to identify, characterize, and map electrophysiologically recorded neurons within a canonical atlas space. This method should prove valuable for structure-function studies where populations of g-s neurons need to be characterized across brain regions or across different physiological conditions.

Disclosures: **E. Peru:** None. **A.M. Santiago:** None. **V.H. Routh:** None. **A.M. Khan:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Keelung Hong Graduate Research Fellowship

Vulnerability Issues in Drug Abuse Graduate Research Fellowship

Title: Further elaboration of forebrain and midbrain neuronal populations projecting to the ventral tegmental area, with an emphasis on the lateral hypothalamic area

Authors: *E. M. WALKER[1,2,3], B. DE HARO[1,3], J. SCHUELER[1,4], R. H. THOMPSON[6], A. M. KHAN[1,6,3,5];
[1]Biol. Sci., [2]Doctoral Program in Pathobiology, [3]Systems Neurosci. Lab., [4]SMART MIND REU

Summer Program, [5]Border Biomed. Res. Ctr., Univ. of Texas El Paso, El Paso, TX; [6]Biol. Sciences, Neurobio. Section, USC, Los Angeles, CA

Abstract: Reward and motivation are essential components of goal-directed behaviors such as eating and drinking. While a central role of the ventral tegmental area (VTA) in reward and motivation is well established, the means by which it interacts with classic hypothalamic feeding circuits is unclear. Here, we have expanded work in our previous report (SfN, Chicago, poster 616.09) to further address this issue. We injected the retrograde tracer Fluorogold (2%; FG) unilaterally into the VTA in 59 adult male Sprague-Dawley rats and mapped the distribution of retrogradely-labeled neurons in the forebrain and midbrain onto the standard atlas templates of Swanson (*Brain Maps: Structure of the Rat Brain, 3rd ed*., 2004). Most retrograde labeling was found ipsilateral to the injection site in all cases; however, a small number of cells was also observed contralaterally at all levels. Small injections largely confined to the VTA retrogradely labeled structures in the tel-, di-, and mesencephalon. *Telencephalic* labeling was found in cortical layers 5 and 6a/b (Levels 25–30), globus pallidus (internal segment), bed nuclei of the stria terminalis, substantia innominata and subfornical organ. *Diencephalic* labeling was present in both the thalamus and hypothalamus. Thalamic labeling was primarily limited to the medial and lateral habenulae, and intermediodorsal nucleus thalamus. In the hypothalamus, most labeling was found in the LHA, although the dorsomedial, ventromedial, paraventricular and posterior hypothalamic nuclei were labeled to some degree as well. LHA labeling was found in the juxtaparaventricular, suprafornical, anteroventral, dorsal, and juxtaventromedial region (ventral zone) parts. *Mesencephalic* labeling was observed primarily in the periaqueductal gray and the medial part of the median superior central nucleus raphé. FG deposits extending beyond the VTA to encroach upon the medial lemniscus and rubrospinal tract resulted in a similar distribution of retrogradely labeled cells in the diencephalon but additionally labeled the nucleus of the posterior commissure, compact and reticular parts of the substantia nigra, medial pretectal area and anterior pretectal nucleus, superior colliculus, red nucleus, oculomotor nucleus, nucleus of Darkschewitsch and midbrain reticular nucleus. These maps contribute to our understanding of the circuit that mediates the interaction between hypothalamic behavioral control systems and those mediating reward and motivation. A better understanding of this interaction and the underlying circuitry is necessary for developing an effective animal model for the treatment of eating disorders and addictive behaviors.

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Poster

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GK-12 Fellowship to AM

UTEP Grand Challenges Award to AMK

Title: Further elaboration of arcuate hypothalamic nucleus circuitry based on retrograde studies in the adult male rat

Authors: *A. MARTINEZ, B. E. PINALES, A. M. KHAN;
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Abstract: The arcuate nucleus (ARH) is an important periventricular neuroendocrine structure. It serves as an active sentinel of metabolic state, contributing critically to the regulation of energy balance. While ARH function has been intensively investigated, a complete understanding of ARH neuronal connections to and from larger network targets remains poorly understood.
To address this issue, we have continued neuroanatomical tract tracing studies of ARH circuitry (SfN 2015 Chicago, poster 616.10/U42) through the use of the retrograde tracer, FluoroGold (FG). FG injections were delivered into the rostral ARH of adult male Sprague-Dawley rats. Brain sections of animals with injection site deposits confined to the ARH, as determined by analysis of adjacent Nissl-stained tissue, were immunohistochemically stained for tracer transport and imaged at high resolution using wide-field epifluorescence microscopy. Retrogradely-labeled FG-positive neurons were plotted onto the Swanson rat brain atlas for a detailed view of the source populations for ARH afferents (Brain Maps: Structure of the Rat Brain, 3rd ed.).
Initial mapping results of an injection case confined to the ARH and approximately 200-300 microns anterior to the injection site dataset presented last year, show a marked reduction of ARH inputs relative to the more caudal site and indicate a stark rostrocaudal difference in ARH afferent input. Preliminary analysis reveals regions with sparse connectivity to the ARH to include the anteroventral preoptic nucleus, preoptic periventricular nucleus, paraventricular thalamic nucleus, and periventricular hypothalamic nucleus; with an absence of retrogradely-labeled neurons (relative to the previous case) in the bed nuclei of the stria terminalis, nucleus accumbens, medial preoptic nucleus, anterodorsal preoptic nucleus, periventricular hypothalamic nucleus posterior part, and ventral premammillary nucleus. While further studies are required to

confirm these differences, these data maps provide the first evidence of heterogeneity in ARH innervation and suggest a possible topography of ARH circuitry that is more complex than previously determined. The high-resolution mapping of these data sets to a standardized rat brain atlas provide detailed information of targetable ARH networks that may be relevant in the regulation of energy homeostasis.

Disclosures: **A. Martinez:** None. **B.E. Pinales:** None. **A.M. Khan:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Support: NIH Grant GM109817 to AMK

RISE Graduate Fellowship to AEH

Title: Migration, spatial alignment, and registration of multi-scale neuroscientific datasets related to the control of motivated behaviors within canonically defined maps of the lateral hypothalamic area

Authors: *A. E. HERNANDEZ[1], A. M. KHAN[2];
[2]Biol. Sci. and UTEP Systems Neurosci. Lab., [1]Univ. of Texas At El Paso, El Paso, TX

Abstract: Addressing how distinct neuronal populations contribute to complex behavior is a collective effort in neuroscience. However multi-scale data generated to address this question have not yet been integrated in a meaningful way. In our laboratory, we seek to *define such meaning* by contextualizing these multi-scale data spatially. We believe that by precisely aligning and visualizing these diverse datasets within single maps, one can both validate and gain insights from current and legacy datasets.
Typically, brain regions where chemicals have been injected to produce a behavioral change are almost never aligned spatially with traced axonal and molecular expression patterns documented to be present in that region. Most data, in the rat, are mapped to either the Paxinos & Watson atlas (*The Rat Brain in Stereotaxic Coordinates*; 'PW') or the Swanson atlas (*Brain Maps: Structure of the Rat Brain*; 'S'). Here, we identified published datasets that have been mapped in PW and then migrated and spatially aligned these data to S in an attempt to integrate multi-scale datasets related to motivated behaviors.
Data mining was performed using online search engines and databases. Retrieved articles

included those with mapped injection sites and molecular expression patterns in the LHA. A total of 32 mapped injection sites were extracted from four articles illustrating regions with anterograde tracer deposits, or injection sites for opioid, glutamate, and dopamine receptor analogs that affected ethanol drinking, food intake, and instrumental conditioning; respectively. Mapped somata were extracted from three articles illustrating hypocretin/orexin (H/O) and neuronal nitric oxide synthase (nNOS) expression after water deprivation, and melanin-concentrating hormone (MCH) and H/O peptide and mRNA expression in the LHA. Using a novel atlas alignment tool, stereotaxic coordinates guided the migration of PW maps to S. PW mapped injection sites for opioid, glutamate and dopamine analogs migrated to S levels 28-30, 29, and 30; respectively. All chemoarchitectural data found across these three levels were placed in register with migrated datasets.
Our visual analysis of mapped data aligned to distinct subdivisions of the LHA provided hypotheses of the relationship among nNOS, H/O, and MCH neuronal populations, their possible co-expression of opioid, glutamate and dopamine receptors, their projections to either the central amygdalar nucleus or to midbrain, hindbrain, and diencephalic regions; and their involvement in behavior. Overall, this study highlights the importance of registering diverse mapped datasets to inform future integrative studies of the LHA.

Disclosures: **A.E. Hernandez:** None. **A.M. Khan:** None.

Poster

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Support: NIH Grant GM109817

UTEP ORSP Grand Challenges Grant

Title: High spatial resolution mapping of Agouti-Related Peptide-immunoreactive axons to a canonical rat brain atlas

Authors: ***B. E. PINALES**[1], J. D. HAHN[2], A. M. KHAN[3];
[1]Biol. Sci., Univ. of Texas At El Paso, El Paso, TX; [2]Biol. Sci., USC, Los Angeles, CA; [3]Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Agouti-related peptide (AgRP) has a well-known role as a neuropeptide involved in feeding control and energy balance. Brain expression of AgRP is predominantly (if not

exclusively) restricted to a subpopulation of neurons in the arcuate hypothalamic nucleus. However, the extensive connections of AgRP-expressing neurons have not been described systematically. Here we used immunohistochemistry to identify AgRP-immunoreactive (-ir) axons, which were then mapped digitally to sequential levels of a canonical rat brain atlas (L. W. Swanson, 2004). Accurate mapping of data to histologically defined gray matter regions was facilitated by reference to Nissl-stained cytoarchitecture, the use of camera *lucida* drawings, and careful determination of plane of section. METHODS: Adult male Sprague-Dawley rats were used in these experiments. Briefly, fixed frozen brain sections were incubated with a rabbit polyclonal antibody raised against the 83-132 amino acid sequence of human AgRP (Phoenix). Labelling was visualized with 3,3'-diaminobenzidine, and the data were mapped with the aid of dark field microscopy. Our initial analysis focused on the forebrain, with data mapped to 13 sequential atlas levels encompassing parts of the cerebral cortex, thalamus, amygdala, bed nuclei of the stria terminalis (BST), and hypothalamus. RESULTS: Regions with the highest density of AgRP-ir axons included the BST, the paraventricular- thalamic (PVT) and hypothalamic (PVH) nuclei, and the periventricular hypothalamic nucleus (PV). Dense AgRP-ir labeling was also noted in the arcuate and dorsomedial hypothalamic nuclei, and the lateral hypothalamic area (LHA). In contrast, labeling was sparse in the anterior- and ventromedial hypothalamic nuclei; regions devoid of labeling included the cerebral cortex, amygdala, most areas of the thalamus (including the anteromedial nucleus and ventral anterior lateral complex), the suprachiasmatic hypothalamic nucleus, and some LHA subdivisions (e.g. anterior region, ventral zone). These data provide an initial series of high-spatial resolution brain atlas maps for AgRP in the rat forebrain. They may be used for comparative analysis with other data (especially data mapped to the same rat brain atlas), and to target interventions precisely to forebrain regions that receive inputs from AgRP-expressing neurons. Further planned analysis will extend the available maps to include additional parts of the brain.

Disclosures: **B.E. Pinales:** None. **J.D. Hahn:** None. **A.M. Khan:** None.

Poster

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Title: Involvement of lateral hypothalamus orexin circuits in cocaine demand

Authors: *C. PANTAZIS[1], E. M. MCGLINCHEY[1,2], G. ASTON-JONES[1];
[1]Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ; [2]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: A hallmark of addiction is high motivation for the abused drug. Addiction-related motivation can be quantified through self-administration behavior under high- and low-effort conditions using a behavioral economics (BE) paradigm. In the BE paradigm, the rate of consumption is measured as the effort required to maintain the desired drug level increases. This generates a demand curve, and the slope of that curve (termed demand elasticity, or α) is inversely proportional to motivation. Previous research from our lab has shown that pharmacological inhibition of orexin receptor-1 signaling during BE performance increases demand elasticity (reduces motivation for cocaine). As orexin (also called hypocretin) neurons in the lateral hypothalamus (LH) have been linked to motivation and drug addiction, we expected that animals with higher motivation for cocaine would have greater activity in LH orexin neurons. Animals were trained on cocaine self-administration and BE, and sacrificed 90 minutes after the point of maximum responding on the demand curve (Pmax). Immunohistochemical staining was used to visualize Fos in orexin neurons, which reflects neural stimulation during the time of maximal effort/motivation (~0 to 30 min before Pmax). We found that Fos activation of orexin neurons in lateral, but not medial or perifornical, hypothalamus correlated with demand elasticity and Pmax. However, contrary to our expectations, the percentage of orexin neurons that were Fos+ positively correlated with demand elasticity, such that high motivation animals (low α) had a smaller percentage of orexin neurons that were Fos+. This may reflect a differential role of orexin in motivation for cocaine at different effort levels, or an interaction between orexin and dopamine (also involved in cocaine motivation). To explore these and other possibilities, we are investigating activities of key inputs and outputs of the orexin system using retrograde tracing and pharmacological methods to elucidate the role of the LH orexin system in cocaine demand. The results of these ongoing studies will be presented, and the role of these orexin circuits in cocaine demand will be discussed.

Disclosures: C. Pantazis: None. **E.M. McGlinchey:** None. **G. Aston-Jones:** None.

Poster

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Title: Methylphenidate reverses attention deficits induced by chemogenetic stimulation of the locus coeruleus in rats performing a 2-alternative forced-choice task

Authors: *M. A. PRESKER, JR[1], E. M. VAZEY[2], J. ZHANG[1], A. SNYDER[2], G. ASTON-JONES[1];
[1]Brain Hlth. Inst., Rutgers - The State Univ. of New Jersey, Piscataway, NJ; [2]Univ. of Massachusetts, Amherst, MA

Abstract: Tonic noradrenergic output of the locus coeruleus (LC) is thought to mediate task engagement. Observational evidence and models of LC function posit that hyper-optimal tone results in a distractible state. Disruptions in norepinephrine (NE) signaling have also been implicated in attention-deficit/hyperactivity disorder (ADHD) and effective pharmacotherapeutics like methylphenidate (MPH) have been shown to exert their cognitive-enhancing effects via altering NE signaling. Understanding the pathophysiology of ADHD has been hampered because there are few accepted rodent models of ADHD and most studies indicating a cognitive enhancing effect of MPH have done so in neurotypical animals. Here we used a 2-alternative forced-choice (2AFC) task and designer receptors exclusively activated by designer drugs (DREADDs) to develop a reversible LC-NE-dependent inattentive ADHD -like behavioral phenotype in rats. We used an AAV with the excitatory hM3Dq DREADD gene downstream of a synthetic dopamine beta-hydroxylase promoter (PRSx8) to selectively express the DREADD and mCherry tag in NE neurons of LC of rats. We stimulated LC-NE neurons specifically by ip injection of the selective DREADD agonist, clozapine-n-oxide (CNO). In control rats, mCherry was similarly expressed without the DREADD. CNO (0, 0.1, 1, 10mg/kg ip) produced dose-dependent deficits in 2AFC task-specific measures of task engagement, attention, and accuracy in LC-hM3Dq animals compared to LC-mCherry controls. LC-hM3Dq activation did not change impulsivity measures during performance of the 2AFC task. When administered following systemic CNO (1mg/kg ip), MPH dose-dependently (0, 0.5, 1 mg/kg ip) reversed the behavioral deficits observed in LC-hM3Dq animals without altering the performance of LC-mCherry controls. These data indicate that hyperactivity of LC results in an inattentive cognitive state, supporting the hypothesis that altered NE function contributes to attention deficits in ADHD. In addition, our findings indicate that low-dose MPH can manage attentional deficits induced by LC hyperactivity. This study describes an LC-specific, dose-dependent, and reversible chemogenetic rat model of inattentive ADHD that can be useful for elucidating the neurobiological basis of this disorder and future directions for corresponding therapeutics.

Disclosures: M.A. Presker: None. **E.M. Vazey:** None. **J. Zhang:** None. **A. Snyder:** None. **G. Aston-Jones:** None.

Poster

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NHMRC Grant 1072706

Title: Intermittent access to cocaine increases demand for cocaine in an orexin/hypocretin-dependent manner

Authors: *M. H. JAMES, C. M. STOPPER, N. E. KOLL, B. A. ZIMMER, G. ASTON-JONES;
Brain Hlth. Inst., Piscataway, NJ

Abstract: ***Introduction*:** Intermittent access (InTA) to cocaine increases motivation for drug on behavioral economic (BE) demand measures (Zimmer et al., 2012). The neural mechanisms underlying these changes in motivation are unclear. The orexin/hypocretin system plays a key role in motivated responding for all drugs of abuse (Mahler et al., 2014). We have previously shown that the selective orexin-1 receptor (Ox1R) antagonist SB-334867 (SB) decreases motivation for cocaine measured with BE in high-demand animals (Bentzley & Aston-Jones, 2015), indicating that the orexin system may drive pathological drug-seeking behavior. Here, we investigated whether changes in motivation for cocaine following InTA occur in an orexin-dependent manner. We also directly compared orexin system function in animals exposed to InTA versus long-access (LgA) to cocaine, an alternative procedure associated with increased motivation for drug. ***Methods*:** Male Sprague-Dawley rats were implanted with jugular catheters for i.v. cocaine self-administration. After initial FR1 self-administration training, rats were trained until stable on a BE procedure to obtain baseline demand values (Bentzley et al., 2013). Rats were then trained for 14d on InTA (5 min access every 30 min for 6h), LgA (6h) or short access (ShA; 1h) FR1 self-administration. Following InTA, LgA or ShA self-administration, animals were again re-stablized on the BE procedure and demand was measured following counterbalanced systemic injections of the Ox1R antagonist SB (0,10,30 mg/kg). Rats then underwent extinction training followed by reinstatement tests with SB. ***Results***: InTA decreased demand elasticity (alpha parameter; increased motivation) and increased the maximum price animals paid for a mg of cocaine (Pmax) compared to ShA. A similar trend was observed following LgA but to a lesser extent than for InTA. In all InTA animals, SB dose-dependently altered cocaine demand towards baseline values. In contrast, SB only normalized demand in animals exhibiting high demand for cocaine following LgA. InTA animals exhibited higher levels of reinstatement compared to both ShA and LgA animals, and this was dose-dependently

attenuated by SB. ***Conclusions*:** InTA to cocaine increases motivation for drug and cued-reinstatement behavior to a greater extent than ShA or LgA, indicating that InTA to cocaine may confer a stronger addiction-like phenotype than LgA. These InTA-induced changes are mediated at least in part by Ox1R signaling. Supported by PHS grant 1-R01 DA006214 NHMRC fellowship 1072706.

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Poster

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Topic: G.02. Motivation

Support: DA006214

DA016511

Title: Attenuating noradrenergic or serotonergic signaling in hippocampus during initial abstinence from cocaine persistently decreases later relapse to cocaine seeking in a sex-dependent manner.

Authors: *A. S. KOHTZ, G. ASTON-JONES;
Neurosci., Brain Hlth. Inst., Piscataway, NJ

Abstract: There are substantial sex differences in cocaine addiction, demonstrated by both clinical and pre-clinical studies. Notably, females are more sensitive than males to stress-induced drug-seeking and relapse. Our lab, and others, have previously shown that the initiation of abstinence (extinction day 1, ED1) represents a stressful event involving abstinence from drug. On ED1, female rats exhibit greater cocaine-seeking behaviors compared to males. We hypothesize that the experience of ED1 can substantially influence later relapse behavior following abstinence. Our prior work shows that locus coeruleus norepinephrine (LC-NE) and dorsal raphe serotonin (DR 5-HT) systems are involved in drug-seeking on ED1. Here, we microinfused a cocktail of 5-HT 1A/1B (WAY100635 plus GR127935) or β1 and β2 adrenergic antagonists (betaxolol plus ICI-118,551) into DH on ED1, and observed drug-seeking behavior on ED1 as well as following 2 weeks of forced abstinence. In males, 5-HT antagonism was more effective in reducing drug-seeking on ED1, whereas β-AR antagonism was ineffective. Following 2 weeks of abstinence males administered 5-HT antagonists showed persistently

reduced drug-seeking, compared to males administered saline or β-AR antagonists on ED1. In females both 5-HT and β-AR antagonism in DH was effective in reducing drug-seeking on ED1, and had persistent effects on drug-seeking following 2 weeks of abstinence. We further examined chemogenetic effects of inhibiting signaling from the DR to the DH via DREADDs, and found similar effects for inhibition of DR-DH signaling to decrease ED1 drug-seeking with persistent effects on later relapse, as in the pharmacological experiments. Our results show that 1) drug-seeking during initial abstinence involves 5-HT and β-AR signaling in female DH, but primarily 5-HT signaling in male DH, and 2) reducing drug-seeking by manipulating 5-HT or β-AR signaling in DH during ED1 can attenuate later relapse. Thus, treatments that modulate 5-HT and β-AR during initial abstinence may facilitate later maintenance of abstinence. This may have significant therapeutic implications for treating drug abuse.

Disclosures: A.S. Kohtz: None. **G. Aston-Jones:** None.

Poster

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Support: DA006214

DA016511

Title: The role of oxytocin neuron activity in drug-seeking during initial abstinence from cocaine self-administration

Authors: *B. LIN[1], A. KOHTZ[2], M. SMITH[3], G. ASTON-JONES[2];
[2]Brain Hlth. Inst., [1]Rutgers Univ., Piscataway, NJ; [3]Neurosurg., Med. Univ. of South Carolina, Charleston, SC

Abstract: The initial abstinence from a drug (Extinction Day 1, ED1) is a critical time point in the progression of addiction that our lab finds can influence later relapse. Prior studies have shown that among females in particular, cocaine seeking behaviors increase significantly during ED1 following a period of cocaine self-administration. Moreover, stress-induced relapse propensity is greater among women, compared to men. Oxytocin (OTC) and corticosterone (CORT) are neurohormones that act as important regulators of the stress response. Oxytocin neurons are primarily found in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus. PVN is separated into three divisions (Rostral, Mid, Caudal) and

projects to central regions of the brain, whereas SON projects to the posterior pituitary gland and regulates peripheral responses. We hypothesize that drug-seeking on ED1 reflects an increase in response to the stress of expected drug, and this may decrease activity of OTC neurons which could contribute to the drug seeking response during ED1. We hypothesized that such drug-seeking may be rescued by exogenous OTC to compensate for lowered OTC activity during ED1. First, we show that female rats exhibit greater increases in CORT on ED1 compared to homecage controls, than do male rats, suggesting that ED1 drug-seeking is indeed a stress-associated behavior. To investigate the activity of OTC neurons during ED1 compared to homecage controls, we stained PVN and SON sections for both Fos and OTC. We show that fewer OTC neurons are Fos active on ED1 compared to homecage controls in a sex and region specific manner. Correlation analyses reveal that rostral PVN may be involved in drug-seeking behavior in males whereas mid-PVN may be involved in drug-seeking behavior in females. Exogenous OTC administration on ED1 decreased drug-seeking on ED1 and when administered during cued-reinstatement. This ED1 treatment, however, did not have long-lasting effects on relapse behaviors following prolonged abstinence. Thus, central OTC signaling is involved in drug-seeking behavior among males and females and may reflect acute stress-dependent mechanisms of drug-seeking.

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Poster

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Topic: G.02. Motivation

Support: PHS Grant R01 MH092868.

Title: A critical role for melanopsin in light deprivation-induced depression

Authors: *H. E. BOWREY, M. H. JAMES, G. ASTON-JONES;
Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Introduction: Research suggests a profound effect of light on mood. In humans, elevated levels of light increase alertness, whereas low light decreases sleep latency. Additionally, a specific type of depression, seasonal affective disorder (SAD), is associated with decreased light availability. In rats, short day-length lighting schedules induce depression and anxiety-like behavior, and chronic light deprivation also leads to a depressive phenotype. Our lab has previously shown that this light deprivation-induced depressive phenotype is due to a locus

coeruleus (LC)-dependent mechanism (Gonzalez and Aston-Jones, *PNAS,* 2008), where light-deprivation induces apoptosis in LC monoamine cells, and loss of noradrenergic-LC cortical fibers in frontal cortex (found also in other animal models of depression). To determine the photic circuit underlying this phenotype, we examined the role of intrinsically photosensitive retinal ganglion cells (ipRGCs) on mood and LC noradrenergic (NA) cell apoptosis, following targeted ablation of ipRGCs.
Method: Male Sprague-Dawley rats received bilateral intravitreal injections of either vehicle (0.01 M PBS; N = 5) or melanopsin-saporin (Mel-SAP; N=4), a targeted toxin that selectively recognizes and eliminates ipRGCs. 10 weeks following intravitreal injections, rats were subjected to behavioral assays of mood (saccharin preference test, elevated plus maze and forced swim test). Rats were then anesthetized and perfused. Brains were sectioned and LC tissue was stained for both Poly ADP ribose polymerase (PARP) and tyrosine hydroxilase (TH, a marker of NA neurons).
Results: Mel-SAP induced a depression-like phenotype as measured by a reduced preference for saccharin and increased immobility during the forced swim test. Mel-SAP had no effect on behavior on the elevated plus maze. Mel-SAP was also associated with increased apoptosis in LC NA cells a seen with increased PARP staining.
Conclusion: These results indicate that loss of ipRGC outflow may induce neural damage in the LC NA neurons, and that this damage is associated with a depressive behavioral phenotype. This has implications for therapy to treat at least some types of depression.

Disclosures: H.E. Bowrey: None. **M.H. James:** None. **G. Aston-Jones:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 453.20/III47

Topic: G.02. Motivation

Support: CIHR MOP-89758

Title: Collateralization of projections from neurons in the PVT to the nucleus accumbens, bed nucleus of the stria terminalis, and central nucleus of the amygdala

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Abstract: The paraventricular nucleus of the thalamus (PVT) is a midline thalamic nucleus with dense projections to the shell of the nucleus accumbens (NAc_{sh}), dorsolateral region of the bed nucleus of the stria terminalis (BST_{dl}) and the lateral/capsular region of the central nucleus of the amygdala (CeA_l). Recent experimental evidence indicates that the PVT is involved in both appetitive and aversive behaviors through the activation of projections to NAc_{sh} and CeA_l. However, a question that has not been addressed is whether the same neurons in the PVT innervate different subcortical targets. This study was done to explore the distribution patterns and the level of collateralization of PVT neurons innervating the NAc_{sh}, BST_{dl} and CeA_l. Accordingly, we performed dual tracing experiments by injecting the retrograde tracers cholera toxin subunit B conjugated to Alexa Fluor-594 or Alexa Fluor-488 in two different subcortical targets in the same rat (different combinations of the NAc_{sh}, BST_{dl} and CeA_l). The number of single and double labeled neurons in the PVT was counted on 50 µm sections every 300 µm through the anterior - posterior extent of the PVT. Adjacent sections were also stained for NeuN to estimate the proportion of neurons in the PVT that were labeled. We found that approximately 60% of the neurons in the anterior portion of the PVT projected to the NAc_{sh} whereas 30% projected to the BST_{dl} and 10% projected to the CeA_l. In contrast, we found that approximately 40% of the neurons in the posterior portion of the PVT projected to the NAc_{sh} while 35% projected to the BST_{dl} and 25% projected to the CeA_l. The level of collateralization in the projections was notable in that nearly half of the BST_{dl} and CeA_l projecting neurons were found to also project to the NAc_{sh}. A similar degree of collateralization was also found in PVT neurons that projected to the BST_{dl} and the CeA_l. It should be noted that the tracer injections were restricted to a relatively small portion of the NAc_{sh} suggesting that the proportion of collateralization reported in this study is likely to be an underestimation. We can also conclude from the observation that a large proportion of neurons in the PVT were labeled following a small injection in the NAc_{sh} that single neurons in the PVT bifurcate extensively in the NAc_{sh} in addition to sending collateral fibers to the BST_{dl} and CeA_l. In summary, the results of the present study indicate a high degree of collateralization of projections from neurons in the PVT to the NAc_{sh}, BST_{dl} and CeA_l. This suggests the potential importance of the PVT in simultaneously coordinating the activity of key regions of the brain involved in mediating emotional and motivational behaviors.

Disclosures: **X. Dong:** None. **S. Li:** None. **G.J. Kirouac:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

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Program#/Poster#: 453.21/III48

Topic: G.02. Motivation

Support: NIDA F31-DA-037680

NIDA R01-DA-038599

Title: Differential activity in the circuitry of the paraventricular nucleus of the thalamus following presentation of an incentive vs. a reward-predictive stimulus

Authors: *J. L. HAIGHT[1], Z. L. FULLER[2], K. M. FRASER[2], S. B. FLAGEL[3,4];
[1]Neurosci. Grad. Program, [2]Undergraduate Program in Neurosci., [3]Dept. of Psychiatry, [4]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: The paraventricular nucleus of the thalamus serves as an interface between cortical, limbic and motor circuits. Recently, there has been increased interest in how the PVT mediates responding to presentation of food- and drug-paired cues. However, the precise role of the PVT and related circuitry in these behaviors has been difficult to identify given that Pavlovian-conditioned cues can act as both predictive and incentive stimuli. Using an animal model that captures individual variation in response to discrete reward-paired cues, we are able to parse these properties of stimulus-reward learning. When rats are exposed to a Pavlovian conditioned approach paradigm, wherein presentation of a discrete cue predicts food reward, some rats, termed sign-trackers (STs), attribute both predictive and incentive value to the cue. These rats will rapidly approach and vigorously interact with the reward-paired cue. Other rats, termed goal-trackers (GTs), treat the cue exclusively as a reward predictor. Upon cue presentation, these rats rapidly approach the location of impending reward delivery. Previous studies from our laboratory have highlighted the paraventricular nucleus of the thalamus (PVT) as a critical component of the neural circuitry mediating these behaviors. Here, we sought to explore the engagement of distinct populations of PVT efferents and afferents in sign- and goal-tracking behaviors. The retrograde tracer flourogold (FG) was injected into either the PVT or the NAc, and levels of cue-induced neuronal activity were assessed by quantifying the amount of c-fos in FG-expressing cells. This technique allows us to assess differences in activation between STs and GTs specifically in afferents to, and efferents from, the PVT. We were interested in examining afferent activity in the medial prefrontal cortex, amygdala, hypothalamus, and ventral subiculum, in addition to activity in PVT efferents to the NAc. Results show that presentation of an incentive stimulus was able to evoke greater activity in PVT afferents from the prelimbic cortex, medial amygdala, and lateral hypothalamus, as well as posterior PVT efferents to the NAc. Interestingly, presentation of a mere predictive stimulus was also able to activate PVT afferents from the prelimbic cortex. Thus, relative to goal-trackers, sign-trackers showed greater engagement of subcortical brain regions; supporting a role for the hypothalamic-thalamic-striatal axis in cue-motivated behavior and specifically in response to incentive stimuli. Further, activity in the PrL to PVT pathway may underlie the processing of the predictive qualities of reward-paired stimuli, regardless of incentive value.

Disclosures: **J.L. Haight:** None. **Z.L. Fuller:** None. **K.M. Fraser:** None. **S.B. Flagel:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Program#/Poster#: 453.22/III49

Topic: G.02. Motivation

Support: Intramural Research Program

Title: Lateral hypothalamus glutamatergic projections to VTA mediate escape responses and aversion in mice

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Abstract: The lateral hypothalamus (LH) is a heterogeneous brain structure that has been classically involved in feeding, motivation and reward. Until recently, the complexity of this structure prevented the study of specific cell populations and their involvement in motivated behaviors. Novel optogenetic approaches has shown the implication of the GABAergic projections from the LH to the ventral tegmental area (VTA) in compulsive-like eating and sucrose seeking. Additionally, glutamatergic projections from LH have also been shown to regulate feeding and to produce aversion-related phenotypes. Nonetheless, the participation of glutamatergic projections from LH to VTA in reward and aversion remains poorly understood. In the present study, we addressed this question by using a combination of behavioral, optogenetic and pharmacological techniques in male VGluT2 mice. We found that VTA photoactivation of glutamatergic LH projections was not reinforcing and significantly reduced feeding in food sated mice. A place conditioning study showed that mice receiving continuous photostimulation (20 Hz) after entering a light paired chamber on a three chamber apparatus developed an aversion for this chamber during training, and also during subsequent testing, when the light was no longer available. The effect was long lasting (at least 35 days) and was also observed using a low photostimulation frequency (2.5 Hz). The behavioral phenotype didn't appear to be caused by an increase in anxiety, as evidenced by open field and defensive burying tests. Indeed, it seemed more related to the escape attempts performed by mice when the pathway was photostimulated. To test this hypothesis, we run a forced-swim experiment: as expected, control mice decreased their performance across time. On the contrary, the experimental mice maintained a constant performance as long as the glutamatergic LH terminals in the VTA were photostimulated. Pharmacological blockade of VTA glutamatergic receptors by administration of AP5 (5 µg/ µl) and CNQX (5 µg/ µl) pointed out to an involvement of NMDA receptors in this phenomenon. Our results demonstrate that activation of glutamatergic fibers originating in the LH and projecting to the VTA are implicated in the regulation of defensive behaviors, such as flight responses, as well as in mediating aversion. They are consistent with a model in which the LH,

given its unique connectivity and neurochemical expression pattern, may convey emotionally relevant information to the VTA, which, in turn, and as a key component of the mesocorticolimbic circuitry, will orchestrate goal-directed behaviors critical for survival.

Disclosures: **M.F. Barbano:** None. **H. Wang:** None. **M. Morales:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Program#/Poster#: 453.23/III50

Topic: G.02. Motivation

Support: NS-23805

Title: Comparison of stimulations of the lateral preoptic area and ventral pallidum using measures of reward, anxiety and ingestion

Authors: *R. A. REICHARD, K. P. PARSLEY, S. SUBRAMANIAN, D. S. ZAHM; Pharmacol. and Physiological Sci., St. Louis Univ. Med. Sch., Saint Louis, MO

Abstract: The roles of the lateral preoptic area (LPO) and ventral pallidum (VP) in influencing motivational state are not clearly understood. We showed previously that stimulation of the LPO increases locomotion but not threat tolerance, assessed as the willingness of animals to enter harsh illumination to obtain sweet pellets. Conversely, stimulation of VP produced only a modest increase in locomotion associated with pivoting, but substantially increased threat tolerance. It is unclear if these behaviors are motivated by reward or anxiety. To address this, effects on reward, anxiety and ingestion of infusions of the GABAA receptor antagonist bicuculline (67ng in 0.25 µl over 60 sec) into the LPO or VP were recorded. Sprague Dawley rats, having received such bicuculline or equivalent saline infusions, were subjected to conditioned place preference (CPP) and elevated plus maze (EPM) testing. Unilateral infusions of bicuculline into the LPO, in addition to robustly stimulating locomotion, were rewarding and anxiolytic, as animals spent more time in the CPP paired chamber during post- as compared to pre-conditioning (520.5s ± 33.7 vs 287.5s ± 30.3, t[11] =-4.262, p=0.001) and spent more time in open arms of the EPM (open/close = 0.594 ± 0.127 vs 0.2024 ± 0.138, t[11]=2.446, p=0.032). The reluctance of LPO infused animals to take sweet pellets in harsh lighting is surprising given these results. Consequently, rats were placed in an activity monitor in dim house lighting and given free choice to consume sweet pellets or normal chow after receiving unilateral infusions into LPO (n=6) or bilateral infusions into VP (n=5) of saline or bicuculline. Bicuculline infusions into the LPO had no effect on ingestion of pellets or chow, whereas infusions into the VP, in addition to

stimulating vigorous pivoting movements, increased consumption of sweet pellets nearly 20-fold (3.63g ± 1.15 vs 0.189g ± 0.0710, t[4]= 3.001,p=0.039), but had no effect on chow consumption. The rigorous pivoting behavior and ingestion observed following VP stimulation co-occurred in all but two cases, leaving open whether two sites are involved. In summary, stimulation of LPO increased a measure of reward, decreased a measure of anxiety, had no effect on ingestion and provided no attraction to sweet pellets in harsh lighting. In contrast, stimulation of VP induced pivoting, ingestion and threat discounting. Evidence regarding to what extent stimulation of VP alters reward or anxiety state is still pending, but preliminary results indicate that rats do not show place preference for VP stimulation.

Disclosures: R.A. Reichard: None. **K.P. Parsley:** None. **S. Subramanian:** None. **D.S. Zahm:** None.

Poster

453. Motivation Neurocircuitry: Thalamus and Hypothalamus

Location: Halls B-H

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Program#/Poster#: 453.24/III51

Topic: G.02. Motivation

Title: Chemogenetic activation of the lateral hypothalamus reverses early life stress-induced anhedonia

Authors: *E. J. CAMPBELL, C. D. ADAMS, C. S. MITCHELL, D. M. HODGSON, C. V. DAYAS;
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Abstract: Early life stress (ELS) is a known antecedent to the development of depression in adulthood. A cardinal symptom of depression is low motivational drive, particularly towards stimuli that are normally naturally rewarding. Maternal separation is a well-established rodent model of ELS precipitating neuroendocrine and behavioral abnormalities in adulthood, similar to what is observed in humans. Non-neuroendocrine cell populations, including those in the lateral hypothalamus (LH), have an important role in arousal and reward status as well as depression-related behaviors. Recently, we showed altered activation of stress sensitive LH cell populations after ELS however, whether these changes also manifest as deficits in motivated behavior is yet to be assessed. Therefore, we examined the effect of ELS (maternal separation) on the motivation to lever press for sucrose reward. Next we sought to test whether chemogenetic (designer receptors exclusively activated by designer drugs, DREADD) activation of lateral hypothalamic circuits could modify sucrose responding in ELS rats. Finally, we characterized the impact of DREADD on orexin and melanin concentrating hormone (MCH) hypothalamic

neuropeptide populations given their importance in reward-seeking.
Male Wistar rat pups (n=52) were removed from dams for 0hrs or 3hrs on postnatal days (PND) 2-14 (No ELS or ELS). On PND 50, rats (n = 23) were bilaterally injected with the hM3D (Gq) DREADD into the LH. On PND 70, all rats were trained to self-administer 10% sucrose during 30min sessions. Following training, rats were tested for motivation to self-administer sucrose during a 90min progressive ratio (PR) schedule of reinforcement once a day for 5 days. On the final PR day, rats were injected (i.p) with either 5% DMSO or 5mg/kg clozapine-N-oxide (CNO) and lever responding recorded. Two hours following the initiation of the final PR test, animals were sacrificed and brains processed for Fos-protein immunohistochemistry.
Our data confirmed that ELS provoked a putative state of anhedonia ie. reduced lever pressing for sucrose under a PR schedule of reinforcement ($p<0.01$). hM3D (Gq) DREADD activation of the LH with CNO recovered this anhedonic behavior in ELS rats ($p=0.04$). This recovery in sucrose-responding was associated with a significant increase in numbers of Fos-positive orexin, MCH and putative GABA neurons in the LH (all p's<0.05).
Our study highlights the importance of the LH in ELS-induced anhedonia. It also provides evidence for the involvement of several neuropeptide populations within the LH in depression-related behaviors following ELS.

Disclosures: **E.J. Campbell:** None. **C.D. Adams:** None. **C.S. Mitchell:** None. **D.M. Hodgson:** None. **C.V. Dayas:** None.

Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

Location: Halls B-H

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Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

NCRR (S10RR014978)

NIH (S10RR031599, R01-NS069696, 5R01-NS060918, U01MH093765)

Title: A multi-modality visualization tool

Authors: ***N. PELED**[1,2], O. FELSENSTEIN[3], R. LAPLANTE[1], T. SITNIKOVA[1,2], S. ZOROWITZ[1,4], A. AFZAL[1,4], A. GILMOUR[1,4], K. K. ELLARD[1,4], D. L. VALLEJO[5], A. C. PAULK[6,2], K. FARNES[6], T. DECKERSBACH[1,4], S. STUFFLEBEAM[1,2], M. HAMALAINEN[1,2], A. S. WIDGE[1,4], S. S. CASH[5], D. D. DOUGHERTY[4], E. N. ESKANDAR[6];

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Abstract: The visualization and exploration of neuroimaging data is important for the analysis of anatomical and functional images and statistical parametric maps. While two-dimensional orthogonal views of neuroimaging data are used to display activity and statistical analysis, real three-dimensional (3d) depictions are helpful for showing the spatial distribution of a functional network, as well as its temporal evolution. For our best knowledge, currently there is no neuroimaging 3d tool which can visualize both MEG, fMRI and invasive electrodes (ECOG, depth electrodes, DBS, etc.). Here we present the multi-modality visualization tool (MMVT). The tool was built for researchers who wish to have a better understanding of their neuroimaging anatomical and functional data. The true power of the tool is by visualizing and analyzing data from multi-modalities. MMVT is built as two separated modules: The first is implemented as an add-on in 'Blender", an open-source 3d visualization software. The add-on is an interactive graphic interface which enable to visualize functional and statistical data (MEG and/or fMRI) on the cortex and subcortical surfaces, invasive electrodes activity and so on. The tool can also be used for a better 3d visualization of the anatomical data and the invasive electrodes locations. The other module is a standalone software, for importing and preprocessing. The users can select the data they want to import to Blender and how they want to process it. The module support many types of analyzed data, like FsFast (FreeSurfer Functional Analysis Stream) and SPM (Statistical Parametric Mapping) for fMRI, MNE (a software package for processing MEG and EEG) raw data for MEG and FieldTrip (MATLAB software toolbox for neuroimaging analysis) data structures for the invasive electrodes. The users can also reprocess raw data using a wrappers for FaFast and mne-python (a python package for sensor and source-space analysis of MEG and EEG data).

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

AAN Clinical Research Training Fellowship to TMH

Fund for Medical Discovery Clinical Fellowship Award to TMH

Title: Representations of aversive risk and decision conflict in the human subthalamic nucleus and globus pallidus internus

Authors: *T. HERRINGTON[1], S. PATEL[2], K. KANOFF[3], S.-T. TSAI[2], S. ZOROWITZ[4], T. SITNIKOVA[4], K. ELLARD[4], T. DECKERSBACH[4], A. WIDGE[5], D. DOUGHERTY[4], E. ESKANDAR[2];
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Abstract: BACKGROUND: Neuropsychiatric symptoms including anxiety, impulsivity, apathy and depression are common in Parkinson's disease (PD) and reduce quality of life on par with the core motor symptoms. Motor symptoms can be alleviated by deep brain stimulation (DBS) of the subthalamic nucleus (STN) or globus pallidus internus (GPi). The STN and GPi are also important nodes in associative and limbic cortical-subcortical networks implicated in impulsivity, anxiety and depression. However, the continuous, high-frequency DBS used to treat movement disorders appears not to help, and may worsen, neuropsychiatric symptoms. In this study we aimed to elucidate the role of the STN and GPi in approach-avoidance behavior, a core neuropsychiatric dimension in parkinsonian depression and anxiety. **DESIGN:** We recorded from single neurons in the human STN (n = 12 subjects, 64 neurons) and GPi (n = 5 subjects, 18 neurons) during clinical microelectrode mapping for placement of deep brain stimulation electrodes. Intraoperative electrocorticography (ECoG) recordings over the DLPFC or SMA/dACC were obtained in a subset of subjects (5 STN, 1 GPi). During recordings patients performed a novel approach-avoidance task that required subjects to accept or reject offers with independently varying levels of reward and aversive risk. **RESULTS:** Single-neuron spike rates in the STN were negatively correlated with aversive risk shortly after the offer, and positively correlated with the decision to approach risk. As subjects prepared to respond, neural responses in the STN were positively correlated with the degree of decision conflict. In contrast, single-neuron responses in the GPi did not correlate with aversive risk, approach-avoidance decision making nor decision conflict. Initial ECoG recordings suggest that theta-band power in the dACC/SMA correlates with approach behavior. **CONCLUSIONS:** Compared to the GPi, the STN may serve a unique role in evaluation of risk and approach-avoidance decision making. This function may involve the medial prefrontal cortex - STN hyperdirect pathway previously implicated in decision conflict monitoring. Alternatively, our STN recordings may have sampled more extensively from the associative and limbic connected subterritories which are in close proximity to the sensorimotor STN DBS target. Recordings from more anterior GPi, which are not obtained clinically, may have revealed greater task engagement. The engagement of the STN in approach-avoidance decision making suggests that novel modes of DBS responsive to the

neuropsychiatric functions of the nucleus could one day help alleviate neuropsychiatric symptoms in Parkinson's disease.

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

Location: Halls B-H

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Program#/Poster#: 454.03/JJJ1

Topic: H.02. Human Cognition and Behavior

Support: W911NF-14-2-0045

Title: Bayesian state-space modeling of reversal learning in fmri

Authors: ***S. ZOROWITZ**[1], A. AFZAL[2], T. DECKERSBACH[2], K. K. ELLARD[2], A. L. GILMOUR[2], D. D. DOUGHERTY[2], E. N. ESKANDAR[3], A. S. WIDGE[2];
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Abstract: Background: Reversal learning is a dynamic process during which associations are undone in light of new, conflicting information. It involves a multitude of neural structures, including dorsolateral prefrontal cortex (DLPFC) and the basal ganglia. Deficits in reversal learning have been implicated in psychiatric disorders including MDD, OCD, and addictive disorders. Conventional approaches to measuring reversal learning underutilize available information, neglect important individual differences, and do not provide per-trial estimates of learning. Here we extend previous work to model per-trial reversal learning in the fMRI.
Methods: 30 healthy participants and 14 MDD/GAD patients performed an fMRI reversal learning task. We devised a Bayesian state-space model (SSM) that estimates a per-trial learning state, informed by response time and accuracy. Time-to-criterion (TTC), or the trial at which a participant achieves and maintains 80% likelihood of correct response after reversal, was calculated for each participant using their individual SSM estimates. Two fMRI models of reversal learning, one with and without the SSM estimates as regressors, were fitted per-subject and compared using paired t-tests.
Results: TTC was longer in patients than in healthy controls ($t=-2.587, p=0.015$), and TTC across participants predicted scores on the BRIEF metacognition scale ($\beta=2.20, p=0.043$) and the BIS-11 nonplanning impulsivity scale ($\beta=0.818, p=0.039$). The fMRI model with SSRE estimates

found greater activation in bilateral DLPFC, dACC, and caudate, and detected deactivation in OFC. Model comparisons show improved SNR gain in DLPFC and caudate with the SSM; OFC deactivation was not detected in the model without SSM estimates.
Conclusions: The SSM model captured variability in reversal learning that is clinically useful, and facilitates more robust detection of activation during reversal learning in the fMRI. The SSM model has great utility in understanding the domain of reversal learning.

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Topic: G.03. Emotion

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Title: Neural stimulation induces changes in behavior and neural responsiveness in the Emotion Conflict Resolution (ECR) task

Authors: A. C. PAULK[1], D. I. VALLEJO-LOPEZ[2], A. DOMINGUEZ[2], N. NOSSENSON[2], N. PELED[3], A. YOUSEFI[1], K. K. ELLARD[4], S. ZOROWITZ[4], A. AFZAL[4], B. CROCKER[2], I. BASU[1], T. SITNIKOVA[3], T. DECKERSBACH[4], D. D. DOUGHERTY[4], E. N. ESKANDAR[1], S. S. CASH[2], *A. S. WIDGE[4];
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Abstract: Modulating psychiatric state along stable therapeutic paths has long been stably driven through counseling and therapy and pharmaceutical approaches. As a supplement to these approaches, we are exploring the use of neural stimulation to tap into and subtly modify how humans respond in one particular psychological domain: emotion regulation. Using a standardized task to sample emotion regulation, the Emotion Conflict Resolution (ECR) task, we both identify the brain regions which are active during the task as well as examine now neural stimulation alters both the behavioral and physiological dynamics during the task. We examine neural activity in the form of local field potentials (LFPs) from a range of brain areas routinely recorded with implanted electrodes during prototypical clinical evaluation of patients with intractable epilepsy (N=9 patients). First, we could correlate ECR task-relevant behavior to the

LFP voltage signal in the dorsolateral prefrontal cortex (dlFPC), the dorsomedial prefrontal cortex (dmPFC), the temporal cortex, the rostral anterior cingulate cortex (rACC) and the orbitofrontal cortex (OFC) ($p<0.001$, N=9, Wilcoxon signed rank test). These same regions exhibited power changes in theta (4-8 Hz) and high gamma (65-200 Hz), with across-patient consistencies such as increased activity in the left hemisphere. Finally, we introduce stimulation in brain regions which were either predicted to be involved in emotion regulation in the fMRI domain or were shown to be active during the task (N=9 patients). We found that 1) left hemisphere stimulation induced the most significant changes in task-relevant behavior ($p<0.0044$, N=9, Wilcoxon signed rank test); 2) stimulation in the amygdala, dmPFC, and dorsal anterior cingulate induced trial-specific behavioral changes across patients; 3) we found a longer term effect of stimulation that altered the LFP responsiveness of specific brain regions to the task which we hypothesize to be dose-dependent (ANOVA, $p<0.000001$). These results point to a measured approach that when and where we stimulate could play a major role in modulating emotional responses to psychologically relevant tasks, with the hope that we can use this information to close the loop between neural activity, neural stimulation, and behavior along therapeutic means.

Disclosures: **A.C. Paulk:** None. **D.I. Vallejo-Lopez:** None. **A. Dominguez:** None. **N. Nossenson:** None. **N. Peled:** None. **A. Yousefi:** None. **K.K. Ellard:** None. **S. Zorowitz:** None. **A. Afzal:** None. **B. Crocker:** None. **I. Basu:** None. **T. Sitnikova:** None. **T. Deckersbach:** None. **D.D. Dougherty:** None. **E.N. Eskandar:** None. **S.S. Cash:** None. **A.S. Widge:** None.

Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

Location: Halls B-H

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Program#/Poster#: 454.05/JJJ3

Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

Title: A biophysical model of electrical stimulation evoked responses in cortical and subcortical brain regions of the human and non human primate

Authors: ***I. BASU**[1], A. C. PAULK[1], K. FARNES[1], M. M. ROBERTSON[1], B. CROCKER[2], D. I. VALLEJO-LOPEZ[2], D. D. DOUGHERTY[3], S. S. CASH[2], E. N. ESKANDAR[1], M. KRAMER[4], A. S. WIDGE[3];
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Abstract: Deep brain stimulation (DBS) is used to treat drug resistant pathologies such as movement disorders, epilepsy, and pain and has potential for managing psychiatric disorders. This works for pathologies affecting a well localized brain region, but is inadequate to treat complex neuro-psychiatric disorders, which are characterized by network-wide alterations and a higher variability across patients. Experimental opportunities with human subjects are too limited to fully understand the complex interactions that arise in the brain network due to stimulation, which has limited the design of new stimulation approaches. To address this challenge, we developed a biophysically relevant model of the cortical and subcortical local field potentials that can be easily parameterized from a limited dataset to computationally explore the impact of stimulation parameters on a patient's brain activity. We used the Jansen-Rit model of single and coupled cortical columns to simulate the effect of electrical stimulation at different frequencies (10-160 Hz) on the local field potential. Each column was modeled as a population of pyramidal cells receiving inhibitory and excitatory feedback from local interneurons and excitatory input from neighboring or distant columns. An additive input to all the populations simulated electrical stimulation. We recorded stimulation-evoked potentials from human subjects undergoing monitoring for epilepsy surgery, then determined a subset of the model parameters by optimizing the correlation between the actual and model response. We also used a dataset recorded from a non-human primate to fit the model and then use data recorded over a period of 2 weeks to test how well the model could predict this data. We found that using a single column model, the simulated evoked responses could model low, but not high frequency stimulation. Including a second coupled column increased the correlation between the simulated and recorded high frequency stimulation evoked responses, especially for sub-cortical regions. The model could also predict evoked responses over a period of a week before it required re-parameterization. This modeling framework can potentially help overcome the inherent drawback in human brain stimulation studies that necessitate a narrow subset of stimulation parameters. By using the model to explore a wide range of stimulation settings, an optimal stimulation strategy for a given patient can be proposed and tested. This has applications in designing DBS-based neurophysiology experiments and in the creation of model-driven closed-loop neuro-stimulation algorithms.

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Poster

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Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

Title: Functional inference distinguishes task and stimulation states across cortical and subcortical networks

Authors: *N. R. PROVENZA[1,3,4], K. FARNES[4], N. NOSSENSON[5], M. M. ROBERTSON[4], D. VALLEJO-LOPEZ[5], A. C. PAULK[4], N. PELED[7], M. W. MCCONLEY[3], S. H. CHIN[3,8], P. D. PARKS, II[3], D. DOUGHERTY[6], S. S. CASH[5], E. N. ESKANDAR[4], A. S. WIDGE[6], D. A. BORTON[1,2];
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Abstract: Closed-loop Deep Brain Stimulation (DBS) may offer a promising alternative therapy for intractable neuropsychiatric illness, including depression, post-traumatic stress disorder, bipolar disorder, attention deficit disorder, substance abuse, and anxiety. However, the neural features of neuropsychiatric illness required to optimally drive stimulation parameters and effectively 'close the loop' remain obscure. The dorsal Anterior Cingulate Cortex (dACC) is associated with cognitive control, attention, and reward-based decision making. Dysfunction in the dACC is prevalent in neuropsychiatric illness, and its associated network is a primary focus for treatment of neuropsychiatric illness with closed-loop DBS. Knowledge of healthy and impaired network connectivity dynamics during periods of high cognitive demand will aid in the development of closed-loop feature classification. In the present study, local field potentials (LFP) were recorded from multi-sensor depth electrodes in epilepsy patients performing the Multi-Source Interference Task (MSIT). The MSIT task is proven to reliably activate the dACC in healthy controls. Canonical correlation and coherence analyses were performed to identify functional connectivity dynamics between pre-identified regions of interest before and during the MSIT task, differentiate between trial types based on functional connectivity, and analyze the effect of in-task DBS on the identified networks. We have found significant differences between MSIT task state and resting state networks, where connectivity during task tends to be greater than during rest. We present preliminary results on how stimulation of cortical and subcortical targets changes MSIT task network dynamics.

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Poster

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Support: Defense Advanced Research Projects Agency (DARPA) under Cooperative Agreement Number W911NF-14-2-0045

Title: An exploration of stimulation effects in the non-human primate brain

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Abstract: Neuropsychiatric disorders are notoriously difficult to treat, due in part to the complexity of heterogeneous networks in the brain. For many years, treatment of mental illness focused on identifying and correcting chemical imbalances across the brain, though such treatments are successful in a limited number of patients and, even then, the side effects can be devastating. Neuromodulation through electrical stimulation may provide an opportunity to finely tailor treatments toward an individual's needs. Recent studies have found success in treating refractory psychiatric illness through deep brain stimulation (DBS), confirming its practicality for treatment. However, traditional DBS methods are limited in that stimulation is constant and often at high frequencies (~130 Hz) regardless of active mental state. Instead, we propose the use of a closed-loop device that will monitor brain activity and stimulate focally in both location and time to alleviate symptoms and shift neural activity in therapeutically relevant directions. Towards this, we examine how single-site and multi-site stimulation at different frequencies and amplitudes alters neural activity in the non-human primate (NHP, N=2) brain. Using chronically implanted miniDBS electrodes that target cortical and subcortical brain regions and an ECoG array placed over the dorsolateral prefrontal cortex (dlPFC), we observe differential effects of stimulation in the ventromedial prefrontal cortex (vmPFC), the dorsal anterior cingulate cortex (dACC), and the nucleus accumbens (NAcc). Each of these targeted regions have been associated with neuropsychiatric disorders, including depression, posttraumatic stress disorder, and addiction. We show that high frequency stimulation in the vmPFC induced changes to the evoked potentials in both the NAcc and the dACC, and also induced a post-stimulation increase in gamma (30-55 Hz) power and suppressed delta (0-4 Hz) power across multiple days (n=5 days). In contrast, dACC stimulation at the same frequencies induced little change in voltage and power. The effects of NAcc stimulation were more complex,

as lower frequency stimulation had comparatively stronger effects on gamma, but this depended on the level of current used. Finally, we examined stimulation-induced changes in coherence across these regions. Taken together, these methods allow us to understand the stimulus space for inducing targeted changes in neural activity. Additionally, we are able to assess the consistency of stimulation across days and months, which is necessary for understanding clinically relevant changes in the effectiveness of DBS in treating neuropsychiatric disorders.

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

Title: Characterizing stimulation-evoked synchrony in brain networks

Authors: ***A. YOUSEFI**[1], A. C. PAULK[1], I. BASU[1], B. NAZARI[4], K. B. FARNES[1], M. M. ROBERTSON[1], B. CROCKER[2], S. S. CASH[2], D. D. DOUGHERTY[3], A. S. WIDGE[3], E. N. ESKANDAR[1], U. T. EDEN[5];
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Abstract: Brain synchrony underlies the formation of functional neuronal networks and mediates their associated functions. Various cognitive and mental brain disorders are related to dysregulated brain synchrony. A major goal in both clinical and basic neuroscience is to characterize dynamics of brain networks and utilize neural stimulation to regularize brain synchrony. Here, we propose a mathematical framework to model synchrony of brain networks. We then use the model to study role of neural stimulation in controlling brain oscillations. State-space methods have been successfully used to model dynamical features of the neural signals. Here, we utilize the state-space framework to describe the dynamics of synchronous activity between two brain areas. We define a state variable that represents the degree of synchrony between two brain areas, and a set of observation processes that characterize the likelihood of measured neural activity in these brain areas as a function of the synchrony state

variable. We also define a state transition process that governs the temporal dynamics of the synchrony state. The neural signals we consider have non-normal distributions, and their relationship with the state variable can be highly non-linear; thus, established state-space algorithms are not able to track state dynamics and estimate model parameters. We develop a new adaptive filter and estimation procedure to track the level of instantaneous synchrony at each instant, while simultaneously estimating the parameters of our model. The algorithm consists of: 1) a filter-smoother procedure, and 2) a Variational-Bayes algorithm, which sequentially estimates model free parameters and the state variable to maximize the likelihood of the observed neural data. We then extend the dynamical model by including additional variables related to neural stimulation. Using this extended model, we are able to characterize the effect of neural stimulation on brain synchrony.
We illustrate this method on neural data collected from both humans and non-human primates. The ultimate goal of this research is to build a new interventional tool for the human patients suffering from brain disorders related to abnormal synchrony.

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Poster

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Topic: G.03. Emotion

Support: DARPA Cooperative Agreement W911NF-14-2-0045

Title: Multimodal exploration of decision-making in the human subthalamic nucleus

Authors: ***S. R. PATEL**, T. HERRINGTON, S. SHETH, M. MIAN, S. BOURNE, S. ZOROWITZ, A. AFZAL, T. DECKERSBACH, A. WIDGE, D. DOUGHERTY, E. ESKANDAR;
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Abstract: Deep brain stimulation (DBS) is being explored for the treatment of neuropsychiatric disease such as Obsessive-Compulsive Disorder and Major Depression. However, the continuously-on motif of first-generation DBS systems may prove to be inefficient in treating more complex and distributed cognitive functions associated with psychiatric disease. We demonstrate closed-loop control of cognitive behavior. In three separate studies, we collected

functional imaging (25 healthy control), single-neuronal recordings from the subthalamic nuclues (STN) undergoing DBS (7 patients), and intermittent STN stimulation behavior (11 patients). For each of these modalities, subjects were engaged in a probabilistic decision-making task. During high-demand trials, we found BOLD activity selectively in the left STN that predicted whether subjects would place a high or low wager ($p < 0.001$; small volume correction). Interestingly, we found that single-neuronal activity in the left STN also predicted high or low wagers during a 500 ms window on a trial-by-trial basis ($F(9,1450) = 2.55$, $p = 0.02$; ANOVA). Finally, we applied intermittent stimulation during this window, selectively on high-demand trials, and found that we were able to make subjects more conservative ($F(2,28) = 2.93$, $p = 0.05$; ANOVA). In this study, we demonstrated that neuronal activity in the STN predicts decisions under uncertainty on a trial-by-trial basis. We then showed that we could apply intermittent electrical stimulation through the implanted deep brain stimulation electrode to bias the decision signal and ultimately alter subject behavior, resulting in less impulsive choices.

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Poster

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Topic: G.03. Emotion

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Title: Oscillatory synchronization enables dynamic information processing to resolve reward seeking vs. risk avoidance conflict

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[1]Dept. of Psychiatry, [2]Dept. of Radiology, Massachusetts Gen. Hosp. & Harvard Med. Sch., Charlestown, MA; [3]Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Optimal cost-benefit analysis is at the core of living a productive and enjoyable life. Deciding to seek a rewarding experience often depends on assessing the involved risks. To understand the neural mechanisms of resolving an approach-avoidance conflict, we concurrently

recorded magneto- and electro-encephalographic (M/EEG) data while healthy subjects (age mean=31, sd=7) decided whether to take varying risk levels of an aversive stimulus in pursuit of varying amounts of a monetary reward. Each trial started with a risk offer (10-90% probability of electric shock to the ankle), which was followed, 1 sec later, with a reward offer (5-95 cents). The risk levels accepted by each subject were median split into low and high perceived-risk conditions. We found evidence that oscillatory synchronization of source localized M/EEG activity reflected differences in information processing between the low and high perceived-risk trials. On the low risk trials, an optimal strategy was to take risk regardless of reward - similar to taking everyday risks of driving a car, regardless of reward value of the trip destination. In contrast, on the high risk trials, it was efficient to postpone the decision until both risk and reward could be considered together - similar to ascertaining the reward value before considering a dicey car drive in a blizzard. We observed that synchrony in the beta rhythm (15-30Hz), previously found to facilitate forming neural ensembles to represent information, was increased, between the ventrolateral prefrontal cortex (VLPFC) and the orbitofrontal cortex (OFC), immediately after the low risk offer, but not until after the reward offer on the high risk trials. Thus, beta synchrony in this circuit, believed to support evaluation of salient information in relation to behavioral choices, may establish neural ensembles to represent the risk, reward, and/or choice information. In addition, synchrony in the alpha rhythm (8-15Hz), previously suggested to suppress irrelevant information, was increased, between VLPFC/OFC and a cognitive control structure, the dorsolateral prefrontal cortex (DLPFC), immediately after the high risk offer, but not until after the reward offer on the low risk trials. This suggested that alpha synchrony may inhibit salient but currently-irrelevant inputs, when the decision is postponed or already made. Individual differences in these temporal patterns of beta and alpha synchrony predicted self-reported abilities to regulate response to emotional stimuli to sustain goal-directed behavior. Synchrony may regulate dynamic representation of the risk, reward, and behavioral choice information during a cost-benefit analysis.

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Poster

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Topic: G.03. Emotion

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Title: Behavioral and neurophysiological dynamics during resolution of cognitive or emotional conflict

Authors: K. B. FARNES[1], *A. C. PAULK[2], D. VALLEJO-LOPEZ[3], M. M. ROBERTSON[1], N. NOSSENSON[3], N. PELED[5], K. K. ELLARD[4], S. ZOROWITZ[4], A. AFZAL[4], T. SITNIKOVA[6], T. DECKERSBACH[4], D. DOUGHERTY[4], E. N. ESKANDAR[1], A. S. WIDGE[4], S. S. CASH[3]; [1]Dept. of Neurosurg., [3]Dept. of Neurol., [4]Dept. of Psychiatry, [2]Massachusetts Gen. Hosp., Boston, MA; [5]Dept. of Radiology, [6]MGH/HST Martinos Ctr. for Biomed. Imaging, Boston, MA

Abstract: Decision making depends on how humans adapt to social and environmental challenges. The neural correlates of flexibility in the face of these challenges has been associated with activity in the cingulo-frontal-parietal cognitive/ attention network (CFP) along with the detection and resolution of emotional conflicts by the salience network. Yet, the intersection of decision and resolving emotional conflict remain largely unknown on the level of populations of neurons, such as what is sampled by local field potentials (LFPs). Using standardized tasks to sample emotion regulation and cognitive flexibility, the Emotion Conflict Resolution (ECR) task and the Multi-Source Interference (MSIT) task, we both identify the brain regions which are active during the tasks through various levels of conflict and correlate these neural dynamics with behavior. We examine LFP from a range of brain areas routinely recorded by implanted electrodes during prototypical clinical evaluation of patients with intractable epilepsy (N=9 patients). First, ECR task-relevant behavior is significantly correlated to the LFP voltage signal in the dorsolateral prefrontal cortex (dlFPC), the dorsomedial prefrontal cortex (dmPFC), the temporal lobe, the rostral anterior cingulate cortex (rACC) and the orbitofrontal cortex (OFC) ($p<0.001$, N=9). Relative to MSIT, ECR induces significantly larger voltage deflections in prefrontal cortical (PFC) and rACC activity across patients ($p<0.001$, Wilcoxon signed rank test). These same regions exhibit power changes in theta (4-8 Hz) and high gamma (65-200 Hz), with increases in activity in the left hemisphere in ECR versus MSIT. In terms of network dynamics, we observe a decrease in coherence between the amygdala and PFC during ECR with a concurrent increase in dACC and PFC coherence during MSIT. We correlate these changes in behavior and physiology to standard psychiatric self-report measures of emotional regulation (ERS, DERS) and cognitive flexibility (BRIEF). These results suggest a wide realm of cortical and subcortical structures are involved in modulating emotional responses in addition to decision making. Yet, specific neural structures, namely the dlPFC, OFC, and rACC, may represent key nodes in differentiating the salience network from the CFP network. We hypothesize how differential network dynamics can explain compensatory neural activation in patients with challenges in emotion regulation or cognitive flexibility. These investigations of spatiotemporal dynamics could help to better understand the processes within psychiatric pathophysiology, informing future clinical innovations in diagnosis and treatment.

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Topic: G.03. Emotion

Title: The control of firing patterns of midbrain periaqueductal gray neurons *In vivo*

Authors: *H. H. SUBRAMANIAN, P. A. SILBURN;
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Abstract: The midbrain periaqueductal gray (PAG) functions as the critical relay of the limbic brain and functions to mediate environmental challenges (flight or freezing) or emotional expression (vocalization, fear, stress or anxiety). In this context, the PAG maintains critical circuitries that control respiration, vocalization, cardiovascular system, pain and analgesia, micturition and, lordosis (Subramanian and Holstege, 2014). However it is not known what type of activity patterns the PAG neurons express in vivo. Using a combination of precollicular decerebrated and, urethane-anesthetized, spontaneously breathing rats the PAG was mapped for extracellular neuronal activity within its various subregions. Drugs were applied by direct microiontophoresis into the PAG to activate silent cells and test their drug sensitivity. Forebrain regions, the anterior cingulate cortex (ACC), hippocampus and amygdala were chemically stimulated to examine their effects on PAG neuronal function. Respiratory, laryngeal and cardiovascular system parameters were monitored during central interventions. Overall the PAG was found to be predominantly quiescent in the resting state. Nonfiring PAG cells could be activated by iontophoresis of the excitatory amino acid glutamate and ceased activity when glutamate ejection was terminated. Few sporadically firing cells were found in the dorsal PAG. These cells fired in a slow, irregular pattern. Robust spontaneoulsy active cells were very few, restricted to the lateral and ventrolateral PAG region. These were either non-bursting cells with a near normal distribution around 200 to 250 msec, or burst-firing cells typically showing a bimodal distribution. However activation of the anterior cingulate cortex, the hippocampus or the amygdala caused immediate activation of PAG neurons. In such instances, PAG cells showed two distinct types of of activity patterns; 1) single spike firing and burst firing, phasic or tonic. The functional implications of PAG neuronal activity are thus discussed in terms of inherent function, inputs from forebrain structures and its impact on descending motor and autonomic control during limbic triggered emotional expression.

References cited: **Subramanian HH** and Holstege G (2014). The midbrain periaqueductal gray changes the eupneic respiratory rhythm into a breathing pattern necessary for survival of the individual and of the species. ***Progress in Brain Research***. 212:352-384.

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Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Topic: G.03. Emotion

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Title: Optogenetic stimulation of the excitatory parabrachial projections to central amygdala induces anxiety- and depression-like behaviors in rats

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Abstract: The amygdala is an important brain area that is involved in integration and modulation of aversive and motivational emotion behaviors including those of affective pain, but the underlying cellular circuitry remains largely unclear. The spinoparabrachial pathway and the projection from the parabrachial (PB) nucleus to the lateral division of central nucleus of amygdala (CeAI) have been characterized as an important cellular circuit that relays pain signal from the spinal cord to the corticolimbic brain for pain processing; however, it is still unknown how the pain signal from this neuronal circuit is perceived and processed to influence integrated emotion behaviors in brain response to pain. In this study, we injected AAV-CaMKII-ChR2-EGFP virus into the PB nucleus to transfect glutamatergic PB neurons and their excitatory projections including those to CeAl. We show here that there are strong direct projections from the glutamatergic PB neurons to CeA and mostly to CeAl. In contrast, no significant PB projection to the neighboring basolateral amygdala is present. In normal rats after PB injection of the virus, photostimulation of the excitatory PB-CeAl projections in CeAI induces significant anxiety- and depression-like behaviors, as measured in the open field test and the forced swimming test, respectively. Interestingly, this photostimulation has no effect on the behaviors of sensory pain in either thermal or mechanical pain test. These findings may suggest that the pain-driven activation of CeAl neurons alone is sufficient to induce negative emotion behaviors of affective pain, an emotion-modulating process that does not influence the perception of sensory pain in acute conditions.

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Poster

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Ruth L. Kirschstein National Research Service Award

Title: Pathway-specific optogenetic manipulations to induce long-lasting changes in anxiety-related behavior

Authors: *A. C. FELIX-ORTIZ[1], G. G. CALHOON[1], A. BURGOS-ROBLES[1], P. NAMBURI[1], K. ANANDALINGAM[2], N. D. BHAGAT[3], K. M. TYE[1];
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Abstract: Anxiety disorders plague up to 28% of adults at some point in their lifetimes, yet existing therapies are burdened with side-effects and can result in increased tolerance and dependence over time with repeated use. We have previously identified the basolateral amygdala (BLA) projection to the ventral hippocampus (vHPC) as a robust pathway that can bidirectionally control anxiety-related behavior upon acute optogenetic manipulation. Given our proven ability to robustly alter synaptic transmission in an acute manner, we next investigated whether we could induce plasticity in such a manner that anxiety-related behavior would be altered in a lasting way. By injecting an anterogradely-traveling viral vector allowing for expression of the stabilized step-function opsin (SSFO) in a cre-dependent manner into the BLA and injecting a retrogradely-traveling viral vector into the vHPC carrying cre-recombinase, we

selectively expressed SSFO in BLA-vHPC neurons. Following 5 days of photostimulation, we observed a decrease in anxiety-related behaviors as measured by the elevated plus maze and open field test at all of the time points we tested, which included 12 hrs, 7 days, 30 days and 90 days following the last optogenetic manipulation. We are now exploring the cellular and synaptic mechanisms underlying this behavioral phenomenon.

Disclosures: **A.C. Felix-Ortiz:** None. **G.G. Calhoon:** None. **A. Burgos-Robles:** None. **P. Namburi:** None. **K. Anandalingam:** None. **N.D. Bhagat:** None. **K.M. Tye:** None.

Poster

454. Neurocircuitry of Emotion: Brain Stimulation and Synchronization

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Title: Negative over positive: unidirectional inhibitory interaction among amygdala projection neurons

Authors: ***G. G. CALHOON**, A. BEYELER, P. NAMBURI, G. GLOBER, K. M. TYE; Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: The ability to anticipate the presence of threats and the availability of natural rewards, and then to use that foresight to select appropriate behaviors is central to survival. The basolateral amygdala (BLA) is a nucleus poised to decipher the predictive value of environmental events, as individual populations of neurons there have been shown to encode cues predicting aversive or appetitive outcomes. We recently identified two largely non-overlapping, projection-target defined populations of BLA neurons which oppositely encode

positive and negative emotional valence. Nucleus accumbens projecting BLA neurons (NAc projectors) undergo synaptic strengthening following reward learning and show a reduction in synaptic strength following fear conditioning, whereas BLA neurons projecting to the centromedial nucleus of the amygdala (CeM projectors) undergo a reduction in synaptic strength following reward learning and enhancement of synaptic strength following fear conditioning. In behaving animals, NAc projectors preferentially encode sucrose predictive cues, whereas CeM projectors preferentially encode aversive quinine predictive cues. However, it is yet unknown how these populations of neurons interact within the BLA. Based upon the opposing roles of these populations in encoding positive and negative valence, we hypothesized that NAc and CeM projectors mutually inhibit each other. To test this hypothesis, we performed targeted whole-cell patch-clamp recordings in BLA projection neurons, identified by the presence of retrogradely travelling fluorescent beads (retrobeads). We then evaluated the impact of photostimulation of the opposing population upon synaptic currents and firing rate in retrobead-positive cells. We found that photoactivation of either population of projectors evokes monosynaptic, glutamatergic currents as well as GABAergic inhibitory currents in the opposing population. Surprisingly, however, we found that photostimulation of NAc projectors *increases* the firing rate of CeM projectors, whereas photostimulation of CeM projectors *inhibits* firing in NAc projectors. These findings suggest that although lateral inhibition exists between both populations, CeM projectors are capable of suppressing activity in the opposing pathway whereas NAc projectors are not. Considered in the context of the individual contributions of these two populations to valence encoding, our present findings provide a potential mechanism allowing fear and escape related behaviors to supersede reward seeking in the presence of threat.

Disclosures: **G.G. Calhoon:** None. **A. Beyeler:** None. **P. Namburi:** None. **G. Glober:** None. **K.M. Tye:** None.

Poster

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Title: A cortico-amygdala circuit encodes observational fear learning

Authors: *S. A. ALLSOP[1], A. C. FELIX-ORTIZ[2], R. WICHMANN[2], A. VIENNE[2], A. BEYELER[2], E. H. NIEH[2], D. BA[3], A. C. SMITH[2], A. EDMONDS[2], A. MAGZOUB[2], E. BROWN[2], K. M. TYE[2];
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Abstract: Observational learning is a powerful survival tool, allowing an individual to learn about environmental stimuli that predict specific threats without direct experience. This ability has been conserved from rodents to humans, and has been linked to the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA). However, little is known about the processes that occur within this circuit, and the degree of specificity to observational learning. To investigate how information is encoded and transmitted through this network, we performed electrophysiological recordings from neurons identified as part of the ACC-BLA network by optogenetic-mediated phototagging to reveal that this network encodes information obtained through observational learning. We also demonstrate that selective inhibition of the ACC-BLA projection impairs observational fear conditioning and other social behaviors, but not classical fear conditioning and that inhibition of ACC input to the BLA alters the amygdalar representation of a cue that predicts shock to another mouse. Lastly, we utilize calcium imaging to assess how ACC neurons involved in observational learning play a role in other social behaviors. Altogether, we show that transfer of cue information from the ACC to the BLA plays a causal role in enabling observational learning and that this same input is needed for general social behavior.

Disclosures: S.A. Allsop: None. **A.C. Felix-Ortiz:** None. **R. Wichmann:** None. **A. Vienne:** None. **A. Beyeler:** None. **E.H. Nieh:** None. **D. Ba:** None. **A.C. Smith:** None. **A. Edmonds:** None. **A. Magzoub:** None. **E. Brown:** None. **K.M. Tye:** None.

Poster

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Topic: G.03. Emotion

Title: Transcranial alternating current stimulation reduced negative emotions and functional integration of anterior cingulate cortex

Authors: *K. ONODA, T. KAWAGOE, H. ZHENG, S. YAMAGUCHI;
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Abstract: Transcranial alternating current stimulation (tACS) is a novel, non-invasive bran stimulation technique. Studies have demonstrated that tACS modulated cortical neural activity affecting perceptual/cognitive performance. However, it is unclear whether effects of tACS reached deep brain areas. We hypothesized that 6 Hz tACS on the frontal area would alter connectivity and network properties of anterior cingulate cortex, based on evidence from frontal theta waves and monitoring responses (error-related, or feedback-related negativity) generated by the anterior cingulate cortex. Resting-state functional MRI (10 min) and emotional state (Positive and Negative Affect Schedule) were assessed before and after tACS (6 Hz, 1000 mA, 10 min). Healthy young voluntary participants (N =22, Mean age = 23.4 ± 3.5 years) were randomly assigned to a real or sham stimulation group. The active electrodes of the tACS were centered around electrode positions F3 and F4 in the 10-20 system. Resting-state functional MRI data were preprocessed by Statistical Parametric Mapping 12 (slice timing correction, realignment, normalization, and smoothing). Effects of white matter, cerebrospinal fluid, and head movements were regressed out from functional data. The whole brain data were divided into 312 regions of interest based on a subdivided automated anatomical labelling template, and mean time courses of BOLD signals were extracted in each region. Functional connectivity matrix for each individual was calculated from correlation coefficients between all pairs of time courses. Measures of functional integration and segregation were computed for each region by applying graph theory to the connectivity matrix. Results indicated that degree and nodal efficiency of the anterior cingulate cortex, which might reflect the level of functional integration, decreased in real, compared to the sham tACS group. Moreover, negative emotion score in real tACS group decreased significantly, whereas positive emotion score decreased in shame tACS group. Importantly, the decreased functional integration of anterior cingulate cortex was significantly correlated with decreased positive emotion scores in the real tACS group. These results suggest that 6Hz tACS on frontal area modulated network properties of anterior cingulate cortex, and caused changes in emotion states.

Disclosures: **K. Onoda:** None. **T. Kawagoe:** None. **H. Zheng:** None. **S. Yamaguchi:** None.

Poster

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University Medical Center Hamburg-Eppendorf Grant NWF 15/06

Title: The neural basis of the human affective startle modulation - evidence from two independent studies using parallel EMG-fMRI

Authors: *M. KUHN[1], J. WENDT[2], R. SJOUWERMAN[1], M. MÖLLER[1], C. BÜCHEL[1], T. B. LONSDORF[1];
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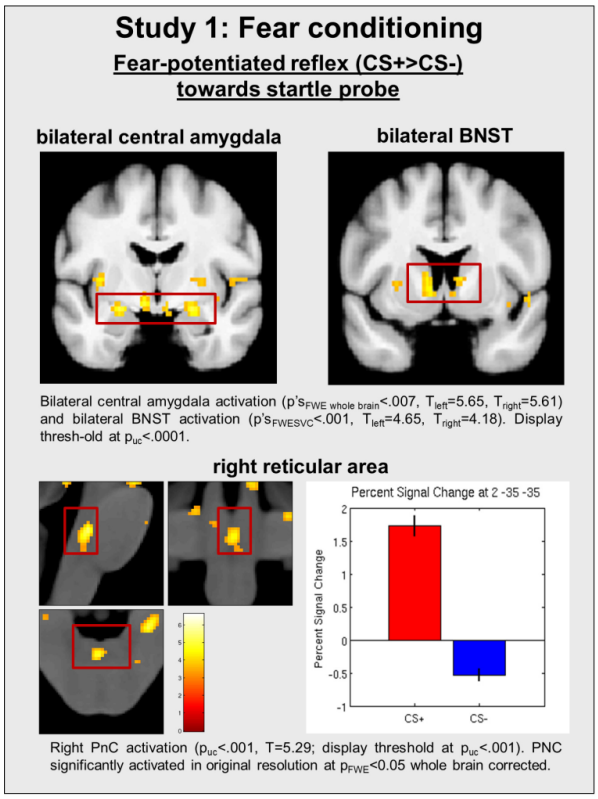
Abstract: Administration of a brief burst of white noise ("startle probe") elicits a defensive reflex cascade which can be measured as whole body startle in rodents and in humans by facial electromyography (EMG) of the orbicularis oculi muscle as a blink reflex. Thereby, response magnitude is modulated by the affective state which manifests as response inhibition in positive and potentiation in negative states.
The neural pathway underlying affective startle modulation has been extensively delineated in rodents and critically involves the nucleus reticularis pontis caudalis and the central nucleus of the amygdala. In humans, this pathway has not been investigated so far even though the startle reflex is widely employed in human emotion ("affective startle modulation") and fear conditioning experiments ("fear-potentiated startle").
For the first time, we provide converging evidence for a similar neural network in humans and rodents by reporting results of parallel facial EMG recordings and fMRI in a fear conditioning experiment (study 1, fear-potentiated startle) as well as an affective startle modulation study using high-resolution brainstem/amygdala fMRI (study 2). We show a relationship between EMG strength and central amygdala activation as well as an affective modulation of the central amygdala and reticular areas of the brainstem. In line with the neurobiological model of affective startle modulation, we further report an increased functional crosstalk between these implicated areas during fear-associated and emotionally negative states. This work has potential impact for translating findings from the vast literature of affective startle modulation in rodents to humans as well as for a more mechanistic investigation of behavioral results on a neural level.



Disclosures: **M. Kuhn:** None. **J. Wendt:** None. **R. Sjouwerman:** None. **M. Möller:** None. **C. Büchel:** None. **T.B. Lonsdorf:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.03. Emotion

Support: NIH Grant R01MH065961

Title: Beta noradrenergic blockade in the basolateral amygdala, but not the medial prefrontal cortex, rescues the immediate extinction deficit

Authors: ***T. F. GIUSTINO**[1], J. R. SEEMANN[2], G. M. ACCA[2], T. D. GOODE[2], P. J. FITZGERALD[2], S. MAREN[2];
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Abstract: Early intervention strategies such as psychological debriefing and exposure therapy are widely practiced following a traumatic experience. These therapies are aimed at reducing the development of stress and trauma-related disorders (e.g., anxiety, phobias, and posttraumatic stress disorder) and are thought to rely on extinction like-processes. However, animal models and human research suggest extinction training often fails to reduce fear long term when administered soon (minutes to hours) after trauma. This is likely due to high levels of psychological stress in the wake of trauma, yielding an immediate extinction deficit (IED). We have previously shown that systemic beta-adrenoceptor blockade (propranolol, 10 mg/kg, i.p.) delivered immediately after fear conditioning (and 30 min prior to immediate extinction training) rescues the IED. In contrast, propranolol administered 30 min prior to delayed extinction (24 hrs post conditioning) produced retrieval deficits. The neural substrates underlying these effects remain largely unknown. Here animals underwent a standard auditory fear conditioning procedure and then received either immediate (30 min after conditioning) or delayed (24 hrs after conditioning) extinction training. We used intracranial infusions of propranolol prior to immediate or delayed extinction targeting either the infralimbic (IL) subdivision of the medial prefrontal cortex or the basolateral amygdala (BLA) to examine the effects of beta adrenoceptor blockade on extinction learning. Interestingly, intra-BLA, but not intra-IL, propranolol rescued the IED; that is, animals receiving intra-BLA propranolol prior to immediate extinction showed less spontaneous recovery of fear during extinction retrieval. In contrast, neither intra-BLA nor intra-IL propranolol modulated delayed extinction learning. Overall, these data contribute to a growing literature suggesting dissociable roles for key nodes in the fear extinction circuit depending on the timing of extinction relative to conditioning. These data suggest heightened noradrenergic activity in the BLA underlies extinction deficits during high psychological stress. Propranolol may be a useful adjunct to therapeutic interventions in recently traumatized individuals who are at risk for developing trauma-related disorders. Supported by NIH grant R01MH065961.

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Poster

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Title: Single neurons in the medial prefrontal cortex of freely moving rats signal fear renewal.

Authors: *P. J. FITZGERALD, T. F. GIUSTINO, S. MAREN;
Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: In Pavlovian fear conditioning experiments, rodents are typically trained to fear a previously behaviorally neutral conditioned stimulus (CS), such as an auditory tone, by pairing it with a footshock. Fear can subsequently be extinguished by presenting the CS repeatedly without shock. Extinction memories are labile and limited to the context in which extinction learning occurred; that is, fear returns when the CS is encountered outside the extinction context (fear renewal). Previous studies suggest the ventral hippocampus plays a critical role in the contextual regulation of fear renewal. However, the role of medial prefrontal cortex (mPFC), a key node in fear modulation, is not well understood in renewal. Here we performed *in vivo* recordings in mPFC of freely moving rats, to investigate single neuron firing patterns during extinction retrieval and fear renewal. In six Long-Evans rats, a microelectrode array was implanted in the right hemisphere, spanning both the prelimbic (PL) and infralimbic (IL) subdivisions of mPFC, to allow for recordings across a five day, modified renewal protocol. Rats were fear conditioned on Day 1 (context A), extinguished (45 tones) in a different context on both Days 2 and 3 (context B), given an unsignaled reminder shock in the conditioning context on Day 4, and finally given a dual retrieval-renewal session, in which the order of these two tests was counterbalanced across animals, on Day 5 (retrieval: context B, renewal: context C). In this design, the same neurons were recorded during both the retrieval (low fear) and renewal (high fear) sessions. Recordings revealed little difference in spontaneous firing between the retrieval and renewal sessions, but the CS-evoked response in IL was lower in renewal than in retrieval. In contrast, PL CS-evoked responses were slightly elevated during renewal. Importantly, individual units showed context-dependent firing to the CS, with 35% of neurons in IL and 28% of neurons in PL showing preferential firing to the CS presented in the retrieval and renewal contexts, respectively. Collectively, these findings suggest that CS-evoked responses in mPFC during renewal convey task-related contextual information. Future recordings in mPFC, combined with chemogenetics, are planned to address potential interactions with ventral hippocampus in transmitting contextual information during fear renewal. Supported by NIH grant R01MH065961.

Disclosures: P.J. Fitzgerald: None. **T.F. Giustino:** None. **S. Maren:** None.

Poster

455. Circuitry and Substrates of Fear

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NIH Grant MH058883

Title: Opposing inhibition in prelimbic prefrontal neurons impairs active avoidance

Authors: *M. M. DIEHL, G. J. QUIRK;
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Abstract: It has been previously demonstrated that pharmacological inactivation of prelimbic prefrontal cortex (PL) with muscimol prevents the expression of platform-mediated avoidance in response to a conditioned tone (Bravo-Rivera, et al., 2014). Last year, we reported that optogenetically silencing PL using Archaerhodopsin (Arch) during the tone delayed but did not prevent avoidance (Diehl et al., 2015 SFN abstract). A possible reason for this discrepancy is that muscimol inactivates all cell types, whereas Arch containing the CaMKIIa promoter preferentially targets glutamatergic neurons. PL neurons recorded during this task showed both excitatory and inhibitory responses to the tone, but only inhibitory responses were correlated with avoidance (Bravo-Rivera, et al., 2014 SFN abstract). PL neurons exhibiting inhibitory tone responses in avoidance were classified as excitatory neurons, based on low baseline firing rate (<15 Hz) and broad spike width (>225 μs; Sotres-Bayon, et al, 2012). This suggests that inhibition of PL glutamatergic neurons may be key for avoidance responses. To test this hypothesis, we used channelrhodopsin (AAV-CaMkII-hChR2(H134R)-eYFP) to prevent tone-induced inhibition of glutamatergic neurons in PL. We stimulated during the tone at a rate of 4Hz, which is the average spontaneous firing rate of PL neurons (Burgos-Robles, et al., 2009). Photoactivation of rostral PL neurons blocked avoidance throughout the tone compared to eYFP controls (rPL-ChR2=17.5% time on platform n=5, eYFP=89.9% n=6, $p<0.01$). In contrast, photoactivation of caudal PL neurons had no effect on avoidance (cPL-ChR2=86.5% n=3, eYFP=89.9% n=6, $p=0.38$). The present findings suggest that inhibitory tone responses in rostral PL are essential for the expression of platform-mediated avoidance. Inhibition of PL glutamatergic neurons could serve to disinhibit neurons in striatum to drive avoidance, similar to disinhibition of basolateral amygdala SOM^+ cells during fear conditioning (Wolff et al, 2014).

Disclosures: **M.M. Diehl:** None. **G.J. Quirk:** None.

Poster

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Support: NIH Grant R01MH065961 to S.M.

Title: Hippocampal-prefrontal projection mediates contextual fear memory retrieval

Authors: *J. JIN[1], T. GOODE[1], Q. WANG[2], S. MAREN[1];
[1]Inst. for Neuroscience; Dept. of Psychology, Texas A&M Univ., College Station, TX; [2]Dept. of Biology, Texas A&M Univ., College Station, TX

Abstract: Extinguished fear to a conditioned stimulus (CS) 'renews' outside of the context in which extinction training occurred. The hippocampus has a critical role in fear extinction, but how it contributes to the context-dependence of extinction memories is not clear. Here we examine the hypothesis that projections from the ventral hippocampus (VH) to the infralimbic region (IL) of the medial prefrontal cortex mediates fear renewal via feed-forward inhibition. In experiment 1, we used 'designer receptors exclusively activated by designer drugs' (DREADDs) to either selectively inhibit or activate VH neurons projecting to IL in different test contexts after extinction. Virus either expressing an inhibitory (AAV8-hSyn-DIO-hM4D[Gi]-mCherry) or an excitatory (AAV5-hSyn-DIO-hM3D[Gq]-mCherry) DREADD was infused into VH, and Cre-expressing virus (CAV2-Cre or AAV5-Cre-eGFP) was infused in the IL to enable DREADD expression in VH neurons projecting to IL. Four weeks later, rats underwent ABA renewal in a counterbalanced within-subjects design. Rats infused with inhibitory DREADD were tested to the CS outside of the extinction context on CNO (1 mg/kg, i.p.). As predicted, DREADD-mediated inactivation of VH neurons projecting to IL disrupted fear renewal as compared to control animals. Moreover, activation of VH neurons projecting to IL by excitatory DREADD led to renewal of fear in the extinction context on CNO (2 mg/kg, i.p.). These data suggest that VH projections to the IL can bidirectionally control fear relapse after extinction. In experiment 2, the hypothesis was further tested by microinfusion of GABA receptor antagonists into the IL during fear renewal. Rats were implanted with a bilateral cannula aimed at IL. A week later, rats underwent ABA renewal in a between-subjects design with microinfusion of $GABA_A$ antagonist picrotoxin, $GABA_B$ antagonist CGP55845, cocktail of two drugs or saline into the IL before renewal test. Consistent with VH-IL circuit manipulation, microinfusions of picrotoxin, CGP55845 or cocktail of two all disrupt fear renewal. These data together suggest that contextual control of fear is mediated by VH-IL circuit via feed-forward inhibition.

Disclosures: **J. Jin:** None. **T. Goode:** None. **Q. Wang:** None. **S. Maren:** None.

Poster

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Topic: G.03. Emotion

Support: NIH Grant R01MH065961 to SM

Title: Reversible inactivation of the bed nucleus of the stria terminalis disrupts the expression of fear to unpredictable threats

Authors: *T. D. GOODE, G. M. ACCA, S. MAREN;
Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: The bed nucleus of the stria terminalis (BNST) is thought to control conditioned fear responses to contexts but not short-duration cues. However, a conditioned context may inevitably differ from a discrete cue in terms of when the animal expects an aversive event to occur during presentation of the stimulus. The current experiments sought to examine whether US predictability is a factor in the recruitment of the BNST, independent of CS duration. First, to establish a strong vs. weak predictor of footshock, rats underwent either forward (i.e., CS-then-US) or backward (i.e., US-then-CS) fear conditioning. Specifically, we hypothesized that the expression of fear to the less predictable (backward) CS would be mediated by the BNST, whereas expression of a forward-trained CS would not. In Experiment 1, rats received twelve forward CS (10 sec, 2 kHz, 80 dB auditory cue)-then-US (2 sec, 1.0 mA footshock) pairings ('FW') or twelve backward US-then-CS pairings ('BW') in Context A (60-sec intertrial intervals, ITIs). Freezing served as the dependent measure of fear. The next day, rats underwent 1 hr of context extinction in the conditioning context or equivalent exposure to a novel context (Context B). 24 hrs later, rats were tested to the auditory CS (eight CS-only trials; 60-sec ITIs) in a separate novel context (Context C). Both forward and backward conditioning resulted in robust fear; fear to the BW but not FW CS was susceptible to extinction of the conditioning context. In Exp. 2, naïve rats were implanted with cannulae targeting the BNST. 1 week after surgery, rats were trained to a FW or BW CS as in Exp. 1, with another group receiving 5 FW conditioning trials instead of 12. 24 hrs after conditioning, rats were infused with the AMPA receptor antagonist, NBQX, or vehicle immediately prior to 12 CS-only testing trials (60-sec ITIs) in a novel context. Intra-BNST infusions of NBQX blocked fear expression to the BW CS, but did not significantly alter fear expression to the 5- or 12-training trials FW CS. A third experiment demonstrated that BNST inactivation disrupted fear expression to a previously conditioned context in which shock (2 sec, 1 mA) was experienced long after placement in the chamber (9 min, 'unpredictable') compared to a context within which shock was experienced soon after

placement (1 min, 'predictable'). These results suggest that fear evoked by unpredictable CSs, whether contexts or cues or long- or short-duration stimuli, are mediated by the BNST.

Disclosures: T.D. Goode: None. **G.M. Acca:** None. **S. Maren:** None.

Poster

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Title: Nucleus reuniens mediates the encoding of extinction memories.

Authors: *K. R. RAMANATHAN[1,2], J. JIN[3], S. MAREN[3];
[2]Dept. of Psychology, [3]Dept. of Psychology and Texas A&M Inst. for neurosciences, [1]Texas A&M Univ., College Station, TX

Abstract: The hippocampus (HPC) and medial prefrontal cortex (mPFC) are two brain areas that have an important role in the extinction of fear memories. Inactivation of the dorsal (Corcoran et al., 2005) or ventral (Sierra-Mercado et al., 2011) hippocampus or the infralimbic cortex (Sierra-Mercado et al., 2011) impairs the acquisition of fear extinction. The anatomy of this circuit suggests that unidirectional, monosynaptic projections from the HPC to the mPFC supports extinction learning. However, the mPFC is positioned to influence information processing in the HPC via indirect projections through the thalamic nucleus reuniens (RE); it is not known how the RE or mPFC->RE projections contribute to extinction learning or retrieval. To address these questions, we first examined whether muscimol inactivation of RE would affect extinction learning. Animals were implanted with a single cannula targeting RE. After recovering from surgery, rats were subjected to 5 tone (CS)-footshock (US) pairings in context A. Twenty-four hours later, they were infused with either muscimol ($GABA_A$ agonist) or saline in RE 10 mins before they received extinction session wherein they received 45 CS-alone trials in context B. This was then followed a day later by retrieval tests in the extinction context (B) and the conditioning context (A). RE inactivation attenuated both within-session extinction, as well as fear suppression in the extinction context the following day; the renewal of fear in the conditioning context was not affected by RE inactivation. To determine if extinction deficits subsequent to RE inactivation are due to blocking PFC input to the RE (and presumably the

HPC), we selectively silenced RE projecting neurons in the mPFC. Rats were first injected with a Cre-dependent inhibitory DREADD (AAV8-hSyn-DIO-hM4D(Gi)-mCherry) in the mPFC and AAV5-CMV-Cre-eGFP in RE to retrogradely transduce DREADD expression in RE projectors in the mPFC. Four weeks later, the rats received the behavioral protocol used in the first experiment. We found that silencing RE projectors in the mPFC reproduced the extinction deficits we observed after muscimol inactivation of RE. Interestingly, there was also stronger renewal in rats extinguished under mPFC->RE inactivation. These results suggest that prefrontal->hippocampal communication is required for fear extinction and the RE is a critical relay to enable this interaction.

Disclosures: **K.R. Ramanathan:** None. **J. Jin:** None. **S. Maren:** None.

Poster

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CNPq

CAPES

FAEPA

Title: TRPV1 receptors modulate aversive responses and social behavior in rodentes

Authors: ***A. B. TERZIAN**, L. RESSTEL;
Pharmacol., Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: TRPV1 receptor (rTRPV1; transient receptor potential vanilloid type 1) is widely distributed in the central nervous system (CNS), on areas involved with modulation of emotional responses such as midbrain and prefrontal cortex. Pharmacological or genetic inhibition of this receptor reduces neuronal activity, resulting in decreased fear and anxiety levels. However few studies investigated the role of rTRPV1 on acquired fear and social behavior, which are highly related to species survival and several psychiatric disorders. Therefore, this study aimed to investigate the role of rTRPV1 in different phases of cued fear conditioned (CFC) and social investigation (SInv) in wild-type (WT) and rTRPV1 knockout mice ($TRPV1^{-/-}$). Mice (C57bl/6 background; 8-12 weeks) submitted to the SInv protocol, were allowed to explore the empty 3-

chamber box (Habituation) for five minutes. On the following phase (Phase 1), animals were either exposed to an empty cage or to one containing a mouse, located in each extreme of the box. Later (Phase 2; 1h or 24h interval), a new mouse was introduced into the empty cage. The time exploring the cages in each phase was evaluated. The CFC protocol was performed in 3 days - on the first day (d0), animals received foot-shock associated with an auditory cue in context A [5 shocks, 0,65mA, 1s; paired with a tone (30s, 1kHz, 70dB)]. Twenty-four hours later (d1), animals were placed in a different context B, where the auditory cue was presented without the foot-shock. On the third day (d2), animals were submitted to the same conditions as in d1. The acquisition (d0), extinction (d1) and extinction learning (d2) were evaluated based on the level of freezing behavior. As a result on SInv, both WT and TRPV1$^{-/-}$ showed preference for the cage containing a mouse on Phase 1, also, both groups spent more time investigating the new mouse on Phase 2 after 1h delay. Interestingly, when Phase 2 was delayed 24h, TRPV1$^{-/-}$ did not exhibit preference for the new mouse. On CFC protocol, the cued fear acquisition was similar for both groups. Nonetheless, on the following days (d1-d2) TRPV1$^{-/-}$ showed reduced freezing behavior, resulting in improved extinction learning when compared to control. In conclusion, our results suggest rTRPV1 involvement in the modulation of learned behaviors with emotional content, suggesting a new approach to future treatment for psychiatric disorders.

Disclosures: **A.B. Terzian:** None. **L. Resstel:** None.

Poster

455. Circuitry and Substrates of Fear

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 455.08/JJJ24

Topic: G.03. Emotion

Support: CAPES

Cnpq

FAPESP

Title: TRPV1 receptor of the ventral portion of medial prefrontal cortex modulates conditioned emotional response: involvement of local glutamatergic and nitrergic system.

Authors: ***D. L. ULIANA**, L. S. ANTERO, S. F. LISBOA, L. B. M. RESSTEL;
Pharmacol. Departament, Sch. of Med. of Ribeirão Preto - USP, Ribeirão Preto, Brazil

Abstract: Transient Potential Vanilloid Type 1 receptor (TRPV1) present in the ventral portion of medial pre-frontal cortex (vMPFC) modulates the conditioned emotional response (CER), in

contextual fear conditioning (CFC). TRPV1 receptor activation has been related with increase behavior and cardiovascular response during contextual re-exposure. Furthermore, the vMPFC NMDA glutamate receptor and neuronal nitric oxide synthase (nNOS) are involved with CER modulation. Possibly, the response induced by TRPV1 involves modulation of glutamate release and NO production. Therefore, this study tests the hypothesis that TRPV1 receptor modulates CER through NMDA activation and NO production. Male Wistar rats (250-270g) with bilateral guide cannula targeted to the vMPFC were first exposed to a chamber during 10 min (habituation). In a second exposure to the same chamber, they received 3 electrical footshocks (0.85 mA, 2 s). 24h later, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. After additional 24h, the behavioral and autonomic responses (mean arterial pressure - MAP, heart rate - HR and cutaneous temperature - CT) were continuously assessed during the 10 minutes of the re-exposition to the same chamber with no footshocks (test session). Vehicle (Saline or DMSO10%) or/and the TRPV1 agonist (capsaicin; 0.1, 1, 10nmol); NMDA antagonist (LY 2 nmol) or nNOS inhibitor (NPLA; 0.04 nmol) were administered in the vMPFC 10 min before the test session. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 171/2014). TRPV1 agonist receptor in the dose of 1nmol increased the time spent in freezing behavior ($F_{3,21}$=11,66; p<0,05, ANOVA, Tukey pos-hoc), and also induced an enhancement of the rise in MAP ($F_{3,21}$=16,64, p<0.05, ANOVA, Tukey pos-hoc), HR ($F_{3,21}$=9,84, p<0.05, ANOVA, Tukey pos-hoc) and the CT decrease ($F_{3,21}$=3,81, p<0.05, ANOVA, Tukey pos-hoc) during the re-exposure. LY and NPLA, were not able to change either freezing behavior (p>0.05) or autonomic response (MAP, p>0.05; HR, p>0.05; CT, p>0.05). However, when administrated previously to the TRPV1 agonist, capsaicin 1nmol, prevented the CER enhancement. The present data demonstrate that the TRPV1 agonist, capsaicin, changed freezing behavior and autonomic response in the re-exposure session, suggesting that TRPV1 receptor in vMPFC modulate CER, in CFC model. And also, this response seems to be dependent NMDA activation and NO production.

Disclosures: D.L. Uliana: None. **L.S. Antero:** None. **S.F. Lisboa:** None. **L.B.M. Resstel:** None.

Poster

455. Circuitry and Substrates of Fear

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Program#/Poster#: 455.09/JJJ25

Topic: G.03. Emotion

Support: FAPESP

CAPES

FAEPA

Title: The expression of contextual fear conditioning involves ACh release and activation of M1-M3 muscarinic receptors/NO/cGMP pathway in the dorsal hippocampus of rats.

Authors: *L. ANTERO, D. L. ULIANA, L. B. RESSTEL;
Pharmacol., Ribeirão Preto Med. Sch. - USP, Ribeirão Preto, Brazil

Abstract: Contextual fear is evoked by re-exposing an animal to an environment previously paired with an aversive stimulus, followed by alterations in behavioral and cardiovascular parameters controlled by the central nervous system. Several studies provide evidence for the involvement of dorsal hippocampus (DH) in contextual fear conditioning. In addition, the cholinergic system in the rats' hippocampus controls conditioning-related behavior, where an increase in acetylcholine (ACh) is associated with defensive behavior retrieval in contextual conditioning. One of the targets of ACh in the DH is the muscarinic receptors, including M1 and M3 receptors subtypes. These receptors facilitate autonomic and behavioral responses associated to threatening situations. Moreover, evidence shows that activation of cholinergic receptors promotes release of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) in the DH, which can modulate behavioral responses during aversive situations. Therefore, the present study investigated the involvement of ACh, muscarinic receptors and the NO/cGMP pathway in the DH, on contextual fear conditioning expression.
Methods: Male Wistar rats (200-250g), implanted with bilateral guide cannulas direct to the DH, were submitted to contextual fear conditioning (3 electric footshocks, 0.85 mA, 2s). After 24 h, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. On the following day, drugs were administered in the DH 10 min before re-exposure to the conditioning chamber. Drugs used were the acetylcholinesterase enzyme inhibitor, neostigmine (0.1 - 3.0 nmol/500nL); M1 and M3 muscarinic antagonist, fumarate (6.0 nmol/500nL); M1 muscarinic antagonist, pirenzepine (6.0 nmol/500nL); NO synthase inhibitor, NPLA (0.01 nmol/500nL); NO scavenger, cPTIO (0.2 nmol/500nL); and soluble guanylate cyclase inhibitor (sGC), ODQ (0.1 nmol/500nL. Freezing and autonomic responses were recorded for 10 min.
Results: The higher doses of neostigmine increased the expression of a conditioned emotional response. This response was prevented by an M1-M3 muscarinic antagonist, NPLA, cPTIO and ODQ. However, pretreatment with the M1 antagonist only prevented the increased autonomic response induced by neostigmine. Moreover, neostigmine produced an increase in NO release in the DH.
Conclusion: Our results show the involvement of the hippocampal cholinergic system in the expression of contextual fear conditioning, via the M1-M3/NO/sGC pathway. Furthermore, our results suggest that DH muscarinic receptors have different roles on the conditioned emotional response.

Disclosures: L. Antero: None. **D.L. Uliana:** None. **L.B. Resstel:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.03. Emotion

Support: NYSTEM

NIMH

HDRF

HHMI Gilliam Fellow

SFN NSP

1RO1MH108623

Title: Subcortical projection-specific control of innate anxiety and learned fear by the ventral hippocampus

Authors: *J. C. JIMENEZ[1], A. GOLDBERG[3], G. ORDEK[2], V. M. LUNA[2], K. SU[2], S. PENA[2], L. ZWEIFEL[4], R. HEN[2], M. KHEIRBEK[3];
[1]Columbia Univ., New York, CA; [2]Columbia Univ., New York, NY; [3]Univ. of California, San Francisco, San Francisco, CA; [4]Univ. of Washington, Seattle, WA

Abstract: The ventral hippocampus (vHPC) has become appreciated for its role in anxiety-related behaviors, serving as a circuit hub that connects cognitive association regions with subcortical structures that directly regulate mood. Although some studies have observed vHPC activity changes in anxiety-related tasks, it is still not understood how diverse vHPC limbic output streams differentially contribute to emotional behaviors. To understand the mechanisms by which distinct vHPC output streams differentially contribute to anxiety-related behaviors, we first determined whether there existed dedicated output streams from vHPC to different subcortical nuclei. Anterograde tracing studies revealed dense terminal innervation of the Basomedial Amygdala (BMA) and Lateral Hypothalamus (LHA), two regions implicated in anxiety, fear, and behavioral responses to stress. Retrograde tracing techniques revealed that vHPC outputs to the LHA and BMA are segregated into two non-overlapping cell populations within vHPC, with distinct laminar organization within the vCA1 subregion. We next determined how these segregated outputs may differentially modulate anxiety-related behavior, employing optogenetic techniques in downstream terminal fields in BMA and LHA. Optical modulation of vHPC-LHA terminals produced aversion and acutely increased anxiety-related behavior without impacting the encoding or retrieval of contextual fear memories. Conversely, vHPC-BMA

terminal modulation impaired the encoding and retrieval of contextual fear memories without altering innate anxiety-related behavior. To understand the mechanisms by which distinct vHPC output streams could contribute to these behaviors, we used miniaturized microscopes to perform cell-type specific calcium imaging *in vivo* in freely behaving mice. This allowed us to visualize vHPC whole-population and projection-specific activity during anxiety-related and learned fear tasks. We found that, at the population level, vHPC activity increased during exploration of innately anxiogenic environments, including the center of the Open Field test and the open arms of the Elevated Plus Maze and Zero Maze, but not to exploration of a novel object. Alternatively, in CFC, re-exposure to a previously conditioned environment did not impact overall rate of transients, rather, increased correlated activity within vHPC and stabilized the ensemble representations of context. Ongoing imaging studies are aimed at determining if the specific vHPC-BMA and vHPC-LHA cell populations are differentially recruited during innate anxiety and learned fear tasks.

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Poster

455. Circuitry and Substrates of Fear

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Topic: F.04. Stress and the Brain

Support: HFSP

MIUR- FIRB

Title: Differential role of the hippocampal & basolateral amygdalar endocannabinoid neurotransmission in the modulation of fear memory retrieval in rats

Authors: B. RUBINO, M. CARLUCCI, E. SANTI, P. RATANO, *P. CAMPOLONGO; Dept. Physiol. and Pharmacol., Sapienza Univ. of Rome, Roma, Italy

Abstract: To date, the understanding of the relative contribution of the principal endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), in the regulation of memory retrieval of stressful experiences is still limited. To address this issue, we investigated the effects induced by pharmacological manipulation of the endocannabinoid signaling in the dorsal hippocampus or in the basolateral complex of the amygdala (BLA). Adult male Sprague Dawley rats were trained in a Contextual or Auditory Fear Conditioning task (CFC and AFC,

respectively). They were bilaterally infused, 60 minutes before retrieval, into the BLA or into the dorsal hippocampal with the FAAH inhibitor, URB597, or the MAGL inhibitor, KML29, respectively increasing endogenous levels of anandamide and 2-AG. In a first experiment, URB597 (3-30 ng/0.2 µl/side) or KML29 (2-200 ng/0.2 µl/side) were infused into the BLA of rats trained in the CFC task. We found that increasing anandamide or 2-AG signalling in the BLA did not influence the retrieval of fear memories. Conversely, infusion of URB597 (10 ng/0.2 µl) into the BLA of rats trained in the auditory version of the task induced an impairing effect on memory retrieval. In a second experiment, URB597 (3-30 ng/0.5 µl/side) or KML29 (2-200 ng/0.5 µl/side) were infused into the CA1 field of the hippocampus; we found that the infusion of KML29 (2 ng/0.5 µl) impaired retrieval of aversive memory in the CFC, but Intra-CA1 infusions of URB597 or of both URB597 and KLM29 in the AFC task did not influence memory retrieval. The cannabinoid receptor antagonist AM251 blocked the impairing effects induced by URB597 on retrieval of cued fear memory or by KML29 on retrieval of contextual fear memory, thus demonstrating that increasing anandamide levels in the BLA or 2-AG levels in the CA1 region of the hippocampus negatively modulates fear memory retrieval through a CB1 receptor dependent mechanism. Our results suggest that the endocannabinoid system is crucially involved in the regulation of retrieval of memory for stressful experiences and it might represent a new therapeutic target for the treatment of neuropsychiatric disorders, such as the Post-Traumatic Stress Disorder.

Disclosures: B. Rubino: None. **M. Carlucci:** None. **E. Santi:** None. **P. Ratano:** None. **P. Campolongo:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.03. Emotion

Support: DGAPA IN204314

CONACYT CB-2013-220173

Title: Effects of intra-amygdaloid injection of the D1 antagonist SCH23390 on the fear/anxiety induced by the exposure to a living cat in rats.

Authors: *E. N. LEVARIO RAMÍREZ, M. CRESPO RAMÍREZ, M. PÉREZ DE LA MORA; Cognitive Neurosci., UNAM, Mexico, D.F., Mexico

Abstract: Amygdaloid DA system participates in the modulation of conditioned and non-conditioned fear. Behaviorally, the intra-amygdaloid infusion of DA D1 agonists and antagonists elicits anxiogenic and anxiolytic effects respectively on conditioned and non-conditioned models of fear/anxiety suggesting an anxiogenic role for the amygdaloid DA D1 receptors. However, these studies have been restricted to relatively simple and ethologically-poor models. The aim of this study was to evaluate the role of amygdaloid DA D1 receptors on fear/anxiety exposing rats to a living adult cat, a real threatening and ethologically relevant model to rodents. Adult male Wistar rats were exposed to a cat in a rectangular plexiglass box having a safe and a danger zone relative to either the presence or absence of the cat separated by a narrow tunnel. The testing procedure consisted of three 10 min video-recorded phases. At the first phase (habituation) rats were allowed to explore the box for 4 days in the absence of any cat. Behavior at the fourth day of habituation was taken as baseline. At the second phase (acquisition), rats received a bilateral intra-amygdaloid injection of either SCH23390 (40 or 120 ng/side), a D1 antagonist or vehicle (saline) and were immediately placed in the safe zone of the box allowing them to explore the whole device, including the danger zone where the cat was already present. At the third phase (retrieval), one day after cat exposure animals were placed into the box but without any cat. Results showed that saline treated rats avoid exploring the danger zone of the box (avoidance behavior) when they are exposed to the cat. SCH23390 infusion into the amygdala has no effects on the time spent by the rats in the danger zone during the acquisition of the avoidance behavior but selectively increased the time spent in this zone following retrieval of the behavior 24 hrs after the exposure to the cat. Our results suggest that amygdaloid DA D1 receptor mechanisms may have a role in the aversive behavior that rats experience in the presence of its predator.

Disclosures: **E.N. Levario Ramírez:** None. **M. Crespo Ramírez:** None. **M. Pérez de la Mora:** None.

Poster

455. Circuitry and Substrates of Fear

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 455.13/JJJ29

Topic: G.03. Emotion

Title: A central amygdala to BNST circuit that regulates anxiety

Authors: *S. AHRENS, B. LI;
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Abstract: The expression of fear and anxiety are evolutionary conserved behaviors that are important for survival as they allow adaptation to a harmful environment by minimizing

exposure to potential threats. The central amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) are important structures in mediating different aspects of fear and anxiety related behaviors and they are highly interconnected. While the amygdala is thought to mediate immediate responses to threat, the BNST is considered to be involved in sustained fear responses.
States of sustained fear often result from an immediate threat; however, how and where this transition occurs is still unclear.
In previous studies we found that cued fear conditioning in mice induces changes in synaptic transmission in the central lateral amygdala (CeL): While the glutamatergic transmission onto Somatostatin positive (SOM+) neurons was increased, the transmission onto Somatostatin negative (SOM-) cells was reduced and this plasticity change was crucial for fear memory. Recently, we found that genetic deletion of the receptor tyrosine kinase ErbB4 in SOM+ neurons causes the same change in synaptic transmission in the CeL and is associated with increased expression of conditioned fear as well as increased anxiety measured in the open field and elevated plus maze. Interestingly, these SOM/ErbB4 mutant mice also display a strongly elevated expression of c-fos of neurons in the oval nucleus of the BNST (ovBNST), a region that receives substantial projections from the CeL and that has been shown to be anxiogenic when activated. An increase in c-fos expression in the ovBNST was also observed in wildtype mice 24h after fear conditioning. This makes the SOM/ErbB4 mutant mice to an attractive model to study the circuits between CeL and ovBNST in fear and anxiety behaviors.
Normalizing the synaptic transmission onto the SOM+ cells in the CeL of ErbB4 mutant mice not only normalizes the expression of conditioned fear, but also reduces the anxiety phenotype and decreases c-fos expression in the ovBNST, suggesting a modulation of ovBNST activity through the CeL.
Electrophysiological recordings in the ovBNST of SOM/ErbB4 mutant mice revealed changes in GABAergic transmission that might provide the underlying mechanism for the increased activity of ovBNST.
Taken together, we propose a model involving a CeL to ovBNST circuit that mediates the transition from an immediate threat response to a sustained fear response, which is important for the animal's behavioral adaptation of risk taking behavior after encountering threatening situations. Disturbances in this circuit are potentially involved in anxiety disorders.

Disclosures: **S. Ahrens:** None. **B. Li:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.03. Emotion

Support: FIRST Program from CSTP, Japan

Title: Netrin-G1 regulates fear and anxiety in dissociable neural circuits

Authors: *Q. ZHANG, S. ITOHARA;
Riken, Wako/Saitama, Japan

Abstract: Netrin-G1 is a glycosyl-phosphatidylinositol-anchored synaptic adhesion molecule whose deficiency results in impaired fear and reduced anxiety under specific circumstance as well. To understand the cell type and circuit specificity of these responses, we generated netrin-G1 conditional knockout mice with loss of expression in cortical excitatory neurons, inhibitory neurons, or thalamic neurons, zona incerta neurons. Genetic deletion of netrin-G1 in cortical excitatory neurons resulted in altered anxiety, but intact fear, whereas loss of netrin-G1 in inhibitory neurons resulted in attenuated fear, but intact anxiety. These data indicate a remarkable double dissociation of fear and anxiety involving netrin-G1 in excitatory and inhibitory neurons, respectively. Our findings support a crucial role for netrin-G1 in dissociable neural circuits for the modulation of emotion, and provide genetic models for investigating the mechanisms underlying the dissociation. The results also suggest the involvement of glycosyl-phosphatidylinositol-anchored synaptic adhesion molecules in the pathogenesis of emotion-related mental disorders.

Disclosures: Q. Zhang: None. **S. itohara:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.03. Emotion

Support: NCN Grant G 2910

Title: Social buffering during fear memory extinction involves medial prefrontal cortex

Authors: *T. GORKIEWICZ, K. ROKOSZ, K. MEYZA, E. KNAPSKA;
Nencki Inst., Warsaw, Poland

Abstract: Social buffering is a phenomenon that has been found in many social species, including humans. It has been observed that presence of a conspecific lowers stress level of

previously stressed animals. Though the behavioral expressions of social buffering are relatively well recognized, little is known on its neuronal underpinnings. Thus, we have developed a model of social buffering in rats that allow for studying neuronal basis of social modulation of fear and its extinction. In this model rats are kept in pairs in home cages for at least two weeks prior to onset of the experiment (to allow them to create stable social bounds). During the first phase of behavioral testing animals are separately fear conditioned. During the next three days one rat from each pair undergoes fear extinction procedure consisting of three sessions a day. The second rat from each pair is at that time only exposed to experimental cage. On the last day of experiment, both animals are tested together. They are placed in the experimental cage, in separate compartments, and presented to conditioned tones. The level of fear (measured as freezing) is compared to the animals treated in the same way but tested alone or rats that were subjected to fear extinction and then tested together. We observed a clear difference in the level of freezing between the rats that were not fear extinguished and tested separately or with a partner. Testing rats together resulted in much lower freezing response than testing them separately, with social exposure as effective in reducing fear as fear extinction. To investigate whether the mechanisms underlying social buffering effect we observed are similar to the ones involved in fear extinction, we compared activation of the prefrontal cortex and amygdala, the structures critical for extinction of conditioned fear. We found lower activation of the anterior cingulate cortex, and prelimbic and infralimbic parts of the prefrontal cortex in animals tested together, whereas no differences were observed in the basolateral and central amygdala. The inhibition of the anterior cingulate and prelimbic cortices resembles the pattern of activation observed in rats subjected to fear extinction; however activation of the infralimbic cortex related to low level of fear after fear extinction was not observed here. Taken together, these results suggest that the mechanisms of social buffering of conditioned fear can only partially rely on the neuronal circuits involved in fear extinction.

Disclosures: **T. Gorkiewicz:** None. **K. Rokosz:** None. **K. Meyza:** None. **E. Knapska:** None.

Poster

455. Circuitry and Substrates of Fear

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Program#/Poster#: 455.16/JJJ32

Topic: G.03. Emotion

Title: Role of dopaminergic D2 receptors of globus pallidus in anxiety response in rat

Authors: G. AVILA[1], E. CHUC-MEZA[2], O. PICAZO[4], *M. GARCIA-RAMIREZ[3]; [1]ENCB-IPN, México, Mexico; [2]ENCB-IPN, Mexico, Mexico; [3]ENCB-IPN, DF, Mexico, Mexico; [4]ESM-IPN, Mexico, Mexico

Abstract: Globus pallidus (GP) is an important nucleus in control of movement. This GABAergic nucleus shows D1 and D2 dopamine receptors and receives dopaminergic innervation from substantia nigra pars compacta (SNc) (Anaya-Martínez et al, 2006; Dejean et al, 2011; Caravaggio et al 2014). In rodents, partial dopaminergic lesion by 6-hidroxydopamine (6-OHDA) in SNc produce anxiety and depression responses (Drui et al, 2014). Additionally, appetitive or aversive stimuli raise dopamine release in GP (Fuchs y Hauber, 2005) suggesting that dopaminergic innervation of GP is also involved in emotive process. In this sense, present work explores participation of dopaminergic innervation of GP in anxiety response of rat. Dopaminergic lesion in GP was made by local administration in both GP with 6-OHDA (500 nL of 6-OHDA, 15 μg/μL). Behavioral tests, elevated plus maze (EPM), burying behavior (BB) and social interaction (SI) and spontaneous motor activity (MA) were performed 30 days after lesion. Results shown reduced time spent in open arms in EPM, increased burying time in BB and decreased social interaction time (SI) without effect in MA. Others groups of rats were implanted with unilateral guide cannula for local administration of 500nL of vehicle, haloperidol (200μM), PD168077 (200μM), amphetamine (90μM) or haloperidol+PD168077. Haloperidol shown similar effects than bilateral lesion with 6-OHDA and were reverted by co-administration of agonist PD168077. In other hand, PD168077 or amphetamine increased time in open arm in EPM and reduced burying time in BB, without change in social interaction time respect of control group. In all cases motor activity was not affected. These results suggest the dopamine innervation in GP, could modulate anxiety behavior acting in D2 receptors. Chuc-Meza and García-Ramírez are COFAA fellow

Disclosures: G. Avila: None. **E. Chuc-Meza:** None. **O. Picazo:** None. **M. Garcia-Ramirez:** None.

Poster

455. Circuitry and Substrates of Fear

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Program#/Poster#: 455.17/JJJ33

Topic: F.04. Stress and the Brain

Title: Histamine in the basolateral amygdala promotes inhibitory avoidance learning independently of hippocampus

Authors: *F. BENETTI[1], C. R. G. FURINI, Furini, CR[2], J. C. MISKYW, Miskyw, JC[3], I. IZQUIERDO[3], E. BALDI[4], G. PROVENSI[4], C. BUCHERELLI[4], M. B. PASSANI[4], P. BLANDINA[4];
[1]Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; [2]Pontifical Catholic Univ. of Rio Grande do Sul, Porto Alegre, Brazil; [3]Pontifical Catholic Univ. of Rio Grande do Sul, porto Alegre, Brazil; [4]Univ. di Firenze, Firenze, Italy

Abstract: Recent discoveries demonstrated that recruitment of alternative brain circuits permits compensation of memory impairments following damage to brain regions specialized in integrating and/or storing specific memories, including both dorsal hippocampus and basolateral amygdala (BLA). Here, we first report that the integrity of the brain histaminergic system is necessary for long-term, but not for short-term memory of step-down inhibitory avoidance (IA). Second, we found that phosphorylation of cyclic adenosine monophosphate (cAMP) responsive-element-binding protein, a crucial mediator in long-term memory formation, correlated anatomically and temporally with histamine-induced memory retrieval, showing the active involvement of histamine function in CA1 and BLA in different phases of memory consolidation. Third, we found that exogenous application of histamine in either hippocampal CA1 or BLA of brain histamine-depleted rats, hence amnesic, restored long-term memory; however, the time frame of memory rescue was different for the two brain structures, short lived (immediately posttraining) for BLA, long lasting (up to 6 h) for the CA1. Moreover, long-term memory was formed immediately after training restoring of histamine transmission only in the BLA. These findings reveal the essential role of histaminergic neurotransmission to provide the brain with the plasticity necessary to ensure memorization of emotionally salient events, through recruitment of alternative circuits. Hence, our findings indicate that the histaminergic system comprises parallel, coordinated pathways that provide compensatory plasticity when one brain structure is compromised.

Disclosures: F. Benetti: None. **C.R.G. Furini:** None. **J.C. MisKyw:** None. **I. Izquierdo:** None. **E. Baldi:** None. **G. Provensi:** None. **C. Bucherelli:** None. **M.B. Passani:** None. **P. Blandina:** None.

Poster

455. Circuitry and Substrates of Fear

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 455.18/JJJ34

Topic: F.04. Stress and the Brain

Title: Cortical drive to the extended amygdala modulates stress-induced behavior in mice

Authors: *K. S. GIRVEN, D. SPARTA;
Anat. and Neurobio. Dept, Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: The ventral bed nucleus of the stria terminalis (vBNST) is a portion of the extended amygdala that has been implicated as a key structure involved in the modulation of fear, stress, and negative affect (Davis, 2010). Although the vBNST receives input from many brain regions, input from the insular cortex (IC) is particularly interesting in relation to stress; being that the IC is a locus for autonomic responses to emotional stimuli (Moraga-Amaro, 2012). We hypothesized that glutamatergic drive from the IC onto vBNST neurons modulate anxiety-like behavior. First, we injected retrograde tracers into the vBNST, and found a dense projection from the caudal portion of the IC. To determine if the IC to vBNST projection promotes aversion and/or anxiety-like behavior we used optogenetics to photostimulate IC terminals in the vBNST during behavior tasks. We then used pharmacogenetics to inhibit IC terminals within the vBNST during a foot-shock stressor. Results implicate this IC-vBNST projection to be critical in the modulation of anxiety-like behavior. Alterations of this circuit may ultimately be important in furthering the modeling of stress related circuitry in the brain.

Disclosures: K.S. Girven: None. **D. Sparta:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: F.04. Stress and the Brain

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NSERC 06106-2015

Title: Neuronal correlates for neuroendocrine adaptation to repeated stress.

Authors: *S. MATOVIC[1], E. W. SALTER[2], X. WANG[2], W. INOUE[1];
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Abstract: Activation of the hypothalamic-pituitary-adrenal (HPA) axis, which results in the increased systemic release of glucocorticoids, is a hallmark of the stress response. The apex of this neuroendocrine response is formed by a group of neurons that synthesize corticotropin-releasing-hormone (CRH) in the paraventricular nucleus of the hypothalamus (PVN). Importantly, this stress response is honed with experience: for example, repeated exposure to a stressor decreases the responsiveness of the HPA axis to that same stressor. Despite its

importance in stress physiopathology, the neuronal plasticity that underlies the adaptation of the HPA axis is poorly understood. Here, we report a neuronal correlate for the adaptation of the HPA axis during repeated stress. We used a CRH-reporter mouse line that expresses red fluorescent protein (TdTomato) in CRH neurons. We challenged these mice (8-12 weeks) with a daily 1h restraint stress for 21 consecutive days. On the 22nd day, repeated restraint mice and control (stress naive) mice received a 1h restraint before dissection. By using immunohistochemistry, we found that stress-induced cFos (a neuronal activation marker) expression in CRH neurons was robustly attenuated in the repeated restraint group compared to control mice, verifying that stress habituation occurs at the level of CRH neurons. In a separate set of mice, we conducted whole-cell, patch clamp electrophysiology from identified PVN CRH neurons and found that repeated restraint stress decreased the intrinsic excitability of CRH neurons. Specifically, we found two mechanistically dissociable changes: 1) the time to elicit an action potential in response to depolarizing current injections was significantly delayed in stressed mice compared to controls; 2) the frequency of repetitive firing during long-duration current injections was decreased by repeated stress. The stress induced change in the delay to first spike was reversed by an A-type potassium channel blocker: 4-aminopyridine (4-AP, 5mM), but was insensitive to a Ca^{2+} chelator (BAPTA, 10mM) included in the internal solution. By contrast, the decrease in repetitive action potential firing was insensitive to 4-AP but was rescued by intracellular dialysis of BAPTA, indicating roles for postsynaptic Ca^{2+} signaling curtaining the excitability of CRH neurons during repetitive firing. Understanding how the HPA axis adapts, or fails to adapt to chronic stress will advance our knowledge about stress resilience, and may lead to the development of new therapeutics, prevention and diagnosis of stress-related disorders.

Disclosures: **S. Matovic:** None. **E.W. Salter:** None. **X. Wang:** None. **W. Inoue:** None.

Poster

455. Circuitry and Substrates of Fear

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 455.20/JJJ36

Topic: F.04. Stress and the Brain

Support: NIH Grant MH105427

Title: C-Fos activation mapping of the bed nucleus of the stria terminalis in response to multimodal stress-inducing stimuli

Authors: ***X. LIN**[1], K. SAMI[1], M. LI[1], F. BERTON[2], W. FRANCESCONI[2], X. XU[1]; [1]Univ. of California Irvine, Irvine, CA; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: The bed nucleus of the stria terminalis (BNST) is an important part of the extended amygdala circuitry, and has been implicated in regulation of acute and chronic stress responses. Given that modern life stresses are often multidimensional and involve multiple concurrent psychological, social and physical aspects, it is of much interest to characterize the pattern of BNST neural activation in the animal model in response to combined or multimodal stress-induced stimuli with concurrent hours-long light, loud noise, jostling and restraint. We also tested the hypothesis that the multimodal stress (MMS) causes strong BNST circuit responses comparable to acute stress with prolonged electrical foot shocks (EFS). In this study, we have assessed neural activation by tracking the expression of the immediate early gene c-Fos in control and stressed mice exposed to either electrical foot shocks (60 shocks within 30 minutes) or multimodal stress (3 hours) at 30-60 minutes, 24 hours, and 1 week following the stress treatment. At each time point, blood samples were collected for plasma corticosterone measurements, and the brains were perfused and sectioned for c-Fos immunostaining. For both stressed groups, we found that c-Fos expression in the BNST regions was abundant at 30-60 minutes following MMS and EFS, and rapidly decreased at 24 hours after the stress treatment. The c-Fos activation one week after was similar to the control level. The stress hormone corticosterone in the blood showed a stress-induced profile temporally correlated to the c-Fos activation strength in the brain. For the MMS group, dorsomedial BNST and ventral BNST regions appeared to have higher densities of c-Fos activated neurons (30-60 minutes: 425 ± 27 /mm^2 and 404 ± 91 /mm^2) than that of dorsolateral BNST (213.93 ± 39.57 /mm^2). For the EFS group, the densities of c-Fos activated neurons for dorsomedial BNST, ventral BNST and dorsolateral BNST regions were 739 ± 34 /mm^2, 515 ± 67 /mm^2 and 302 ± 23 /mm^2, respectively. Both MMS and EFS also caused high concentrations of acute c-Fos expression in other brain regions including the prefrontal cortex, olfactory cortex, thalamus, paraventricular nucleus of hypothalamus, and central amygdala. Taken together, our data support that the BNST is critically involved with acute stress responses including MMS and EFS, and that MMS appears to be as effective as EFS in stress induction.

Disclosures: **X. Lin:** None. **K. Sami:** None. **M. Li:** None. **F. Berton:** None. **W. Francesconi:** None. **X. Xu:** None.

Poster

455. Circuitry and Substrates of Fear

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Program#/Poster#: 455.21/JJJ37

Topic: F.04. Stress and the Brain

Support: MH-095972

Title: Distinct neural projections from the anteroventral bed nuclei of the stria terminalis modulate the endocrine and behavioral stress responses

Authors: *S. B. JOHNSON[1], R. M. ANDERSON[1], E. B. EMMONS[2], S. A. ROMIG-MARTIN[1], N. S. NARAYANAN[2], R. T. LALUMIERE[1], J. J. RADLEY[1];
[1]Psychology, [2]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: The bed nuclei of the stria terminalis (BST) are critically important for integrating stress-related signals between the limbic forebrain to hypothalamo-pituitary-adrenal (HPA) effector neurons in the paraventricular nucleus of the hypothalamus (PVH). Nevertheless, the circuitry underlying BST control over the HPA axis and stress-related behaviors has remained obscure. To better understand these functions, we employed optogenetic techniques to manipulate activity in the somata and terminal fields of the anteroventral (av) BST. Adult rats received bilateral microinjections, into avBST, of adeno-associated viruses expressing channelrhodopsin (ChR2[E123A]-eYFP; ChR2), archaerhodopsin (eArch3.0-eYFP; Arch), or a control virus (YFP) under the synapsin promoter. Photoexcitation of avBST cell bodies during 10 min tail suspension abrogated HPA output (by 25% corticosterone [CORT], 10 min after stress onset; $p < 0.05$), while photoinhibition augmented ACTH abd CORT levels (by XX% and 30%, respectively, 30 min after stress onset; $p < 0.05$ for each) and increased immobility (by 32%; $p < 0.05$). Subsequent experiments interrogated terminal fields emanating from the avBST to account for the endocrine and behavioral functions subserved by this cell group. These experiments identified that the PVH and ventrolateral periaqueductal gray (vlPAG) as recipients of functionally distinct GABAergic avBST outputs capable of restraining stress-induced HPA activation and behavioral coping, respectively, during stress exposure. Specifically, photoinhibition of avBST terminals in PVH augmented HPA output (both ACTH and CORT at 30 and 60 min after stress onset; $p < 0.05$ for each) without affecting immobility behavior. Conversely, photoinhibition of the avBST-vlPAG pathway had no effect on HPA output, but significantly augmented immobility in both the tail suspension and forced swim tests (by 150% and 51%, respectively; $p < 0.05$ for each). These results direct attention to the avBST as a region important for the adaptive coordination of endocrine and behavioral output, identifying a novel circuit wherein impairment could account for core features of stress-related mood disorders.

Disclosures: **S.B. Johnson:** None. **R.M. Anderson:** None. **E.B. Emmons:** None. **S.A. Romig-Martin:** None. **N.S. Narayanan:** None. **R.T. LaLumiere:** None. **J.J. Radley:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA029091

NIDA Grant T32 DA007287

Title: Topographic gene expression analysis of the nucleus accumbens shell and ventral tegmental area yields novel therapeutic target possibilities for anxiety, depression, and drug addiction

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Pharmacol. and Toxicology, Ctr. for Addiction Res., Univ. of Texas Med. Br., Galveston, TX

Abstract: Psychiatric disorders such as addiction have proven difficult to treat and the available pharmacotherapies for anxiety and depression are not effective for all individuals or have unwanted side effects. Novel target identification strategies are therefore necessary to develop effective pharmacotherapeutics for these disorders, especially with minimal side effects. One strategy is to select targets based on gene expression patterns. Genes have evolved to be expressed to a large degree only in the specific cells they are needed. We hypothesized that genes with specific topographical expression patterns in the reward circuitry, specifically the nucleus accumbens shell (NAcSh) and the ventral tegmental area (VTA), would provide novel targets/pathways that would control behavior. Therefore we conducted a topographic gene expression analysis of with the Anatomic Gene Expression Atlas (AGEA) from the Allen Brain Atlas. Gene expression lists for the VTA and NAcSh were then analyzed for pathway relationships with Ingenuity Pathway Analysis. Some genes with expression restricted to the NAcSh are fatty acid binding protein 5 (FABP5), cocaine and amphetamine regulated transcript preopropeptide (CARTPT), cytochrome P450 family 26b polypeptide 1 (CYP26B1), and activating transcription factor 3 (ATF3), for which we and others have validated in addiction and depression studies. In addition to the expected pathways Glutamate Receptor Signaling and cAMP-mediated Signaling, novel pathways in the NAcSh included Cardiac Beta-adrenergic Signaling, Relaxin Signaling, and tRNA Splicing. Top upstream regulators in the NAcSh included cAMP responsive element binding protein 1 (CREB1), huntingtin, and L-dopa. Genes identified with specific expression in the VTA included dopa decarboxylase (DDC), tyrosine hydroxylase (TH), alpha synuclein (SNCA), and neuron-derived neurotrophic factor (NDNF). Top canonical pathways identified in the VTA included Glutamate Receptor Signaling and Dopamine Receptor Signaling but also Cholesterol Biosynthesis, Flavin Biosynthesis, and LXR/RXR Activation. Top upstream regulators included histone deacetylase 4 and brain derived neurotrophic factor (BDNF). MicroRNAs previously found to be altered in the blood of depressed patients were also identified as upstream regulators in the NAcSh (miR-17-5p) and the VTA (miR-34a-5p). Through a topographic gene expression analysis we have identified novel targets in the VTA and NAcSh, some of which have been validated. Therefore novel target possibilities for developing therapeutics for depression and addiction can be identified with topographic analysis of gene expression patterns.

Disclosures: **E.J. Crofton:** None. **Y. Zhang:** None. **S. Koshy:** None. **T.A. Green:** None.

Poster

455. Circuitry and Substrates of Fear

Location: Halls B-H

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Program#/Poster#: 455.23/JJJ39

Topic: F.04. Stress and the Brain

Support: R25NS08068

Title: Modulation of the endocannabinoid system within the nucleus accumbens shell elicits anxiolytic-like effects in rats

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Abstract: At the moment, the functional role of the co-localization of the cannabinoid type-1 (CB1) and the transient receptor potential vanilloid type-1 (TRPV1) receptors within the Nucleus Accumbens shell (NAc shell) on anxiety states has not been studied. Thus, the present study seeks to elucidate the effects of this receptor interaction within the NAc shell on anxiety states. We hypothesized that blocking TRPV1 and the fatty acid amide hydrolase (FAAH) within the NAc shell would elicit an anxiolytic response in rats. In the present experiment, male Sprague Dawley rats were implanted with bilateral brain cannula aimed at the NAc shell. Following recovery from surgery, rats received pre-treatment of microinfusions (0, 0.125, 0.5 nmol/0.4 µl) of N-arachidonoyl-serotonin (AA-5-HT), a dual blocker of FAAH and TRPV1, within the NAc shell. After treatment, rats were tested in an elevated plus maze paradigm for a period of 5 minutes. Behavioral parameters measured were: time spent in open arms, time spent in closed arms, rearing, flatback and grooming. At the end of the experiment, rats were euthanized and their brains collected for histological and western blot analysis. Results showed that pre-treatment with both doses of the antagonist significantly increased the time spent in the open arms and decreased the time spent in the closed arms when compared to vehicle injections ($p < 0.0001$ for both doses and parameters). Furthermore, western blot analysis of rats pre-treated with 0.5 nmol/0.4 µl of AA-5-HT revealed CB1 receptor downregulation in the NAc shell. The present findings suggest that the endocannabinoid system modulates anxiety within the NAc shell, giving this area a more important role in the regulation of anxiety than previously thought.

Disclosures: **T. Pardo:** None. **N. Yusif:** None. **C.S. Maldonado:** None.

Poster

455. Circuitry and Substrates of Fear

Location: Halls B-H

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Program#/Poster#: 455.24/JJJ40

Topic: F.04. Stress and the Brain

Title: The anxiolytic effect of elevated 2-Arachidonoylglycerol signalling in the basolateral amygdala is mitigated by heightened levels of emotional arousal

Authors: *K. LEITL[1], M. MORENA[1], H. VECCHIARELLI[1], M. GRAY[1,2], P. CAMPOLONGO[2], M. HILL[1];
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Abstract: Endocannabinoid (eCB) system manipulation can induce distinct and often opposite effects on anxiety, cognition depending on the stress level and the aversiveness of the environmental context. The basolateral complex of the amygdala (BLA) represents an important structure for the ability of eCB signaling to modulate emotional behavior, and the target of brainstem projections that modulate arousal. We recently reported that high levels of emotional arousal were associated with an impairment in the anxiolytic effects of elevating anandamide (AEA) signaling in the BLA. To extend these findings, we examined the impact of 2-arachydonoylglycerol (2-AG) manipulations within the BLA on anxiety-like behavior and determined if these effects were sensitive to changes in the animals' arousal state. Male adult Sprague-Dawley rats were tested for anxiety behavior in an Elevated Plus Maze (EPM) for 5 min under low or high arousal conditions. The low arousal (LA) group was extensively handled and habituated to the experimental room and tested under red light condition, the high arousal (HA) group was not handled or habituated and tested under high light condition. We have previously found increased AEA levels in the amygdala, selectively under LA conditions and a positive correlation between increased AEA levels and anxiolytic-like behavior shown during the EPM. Moreover, pharmacologically-induced elevation of AEA signaling locally in the BLA further reduced anxiety in LA animals through the activation of cannabinoid type 1 (CB1) receptors, without being effective in the mitigation of the arousal-induced anxiety response in the HA group. With regard to 2-AG, we found that neither of the two different arousal conditions altered 2-AG levels in the amygdala, however we surprisingly found that 2-AG levels positively correlated with higher levels of anxiety. In the second part of this study we evaluated the effects of intra-BLA infusion of the 2-AG hydrolysis inhibitor KML29 and the CB1 receptor antagonist AM251 on anxiety behavior. HA and LA rats were given bilateral intra-BLA administration of KML29 (200 ng/side) or its vehicle alone or together with a non-altering behavioral dose of AM251 (1 ng/side) 30 min prior to the EPM test. Similar to what was found with AEA signaling, we found that KML29 was able to selectively decrease the anxiety response in LA rats through a CB1-mediated mechanism, without affecting emotional behavior in the HA group. Together,

these findings suggest that, depending on the arousal state, the eCB system is differentially activated to regulate the anxiety response in the amygdala and help to understand the state-dependency of many interventions on anxiety.

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Poster

455. Circuitry and Substrates of Fear

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Program#/Poster#: 455.25/JJJ41

Topic: G.03. Emotion

Title: ASIC1a in ASIC4-positive neurons is important for innate fear and anxiety phenotypes.

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[1]Academia Sinica/Institute of Biomed. Sci., Taipei, Taiwan; [2]Inst. of Biomed. Sciences, Academia Sinica, Taipei, Taiwan; [3]Taiwan Mouse Clinic, Natl. Comprehensive Mouse Phenotyping and Drug Testing Center, Academia Sinica, Taipei, Taiwan

Abstract: ASIC4 is a member of acid-sensing ion channels and widely expressed in many nuclei in the CNS. However, the physiological function of ASIC4 is still unclear. To probe the role of ASIC4, we generated an ASIC4-knockout/Cre-ERT2-knockin mouse line. Compared with wild-type littermates, ASIC4 knockouts showed higher levels of fear response (freezing) to the presence of 2,4,5-trimethylthiazoline (TMT), a component of fox urine; ASIC4 knockouts also demonstrated higher levels of anxiety-like behaviors in the open field (OF) and elevated plus maze (EPM) task. These phenotypes were opposite to the ASIC1a knockout mice, which showed lower levels of TMT-induced fear and less anxiety-like behaviors in these two mouse anxiety tasks. Based on genetic mapping results, we hypothesized that ASIC4 in the CR/VIP-positive cortical interneurons (or the NG2-positive glia) might modulate innate fear and anxiety state by counteracting the membrane expression and thus the activity of ASIC1a. To test this working hypothesis, we first generated $ASIC4^{CreERT2/+}$::$ASIC1a^{f/f}$ conditional knockouts and screened the phenotypes in the TMT, OF and EPM task. Results indicated that $ASIC4^{CreERT2/+}$::$ASIC1a^{f/f}$ conditional knockouts showed ASIC1a-like phenotypes in the innate fear and anxiety tests. We further examined whether ASIC4 KO affects the ASIC1a protein expression in specific brain areas. Interestingly, we found ASIC1a membrane protein expression is increased in ASIC4 KO in PAG, BNST, pituitary gland, VPM/VPL, amygdala and cerebellum as compared with wildtype control. Our results supported that ASIC1a in ASIC4-positive neurons is responsible for the fear/anxiety phenotypes.

Disclosures: **Y. Chien:** None. **S. Lin:** None. **C. Chen:** None.

Poster

455. Circuitry and Substrates of Fear

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01DA011289

NIH Grant K99MH106757

NARSAD Young Investigator Award

Title: Circuit-specific plasticity of inhibitory synapses on VTA dopamine neurons

Authors: ***K. BARCOMB**, A. M. POLTER, A. C. TSUDA, J. A. KAUER;
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Abstract: GABAergic synapses are crucial regulators of the excitability of VTA dopaminergic neurons. Our previous work has shown that these synapses undergo nitric-oxide dependent long-term potentiation (LTP_{GABA}) that is sensitive to both drugs of abuse and stress (Polter and Kauer, EJN, 2014). It remains unknown, however, whether this phenomenon is specific to certain circuits within the complex structure of the VTA. GABAergic synapses in the VTA arise from both local neurons and from long-range projections from the rostromedial tegmental nucleus (RMTg), and nucleus accumbens (NAc), among others. Furthermore, dopaminergic neurons within the VTA are heterogeneous, with distinct populations projecting to the NAc and the prefrontal cortex. While this region has been highly studied, plasticity and cell-type specific properties of its inhibitory circuitry are still not well understood. Given the importance of VTA function in disorders such as substance abuse and depression, a better understanding of plasticity within inhibitory subcircuits of the VTA and how it is modulated by stress may reveal circuit-level mechanisms of stress-linked diseases.

Here, we use viral-mediated expression of channelrhodopsin and retrograde microbead labeling to test the circuit specificity of LTP_{GABA} induced by the nitric oxide donor, SNAP. We find that synapses arising from local VTA GABAergic neurons express LTP_{GABA}. Synapses from local GABA neurons on both putatively dopaminergic (Ih+) and non-dopaminergic (Ih-) neurons displayed similar average magnitudes of LTP_{GABA} (LTP magnitude, Ih+: 130 ±9% of baseline, n=15; Ih-: 126 ±11% of baseline, n=12). However, a smaller proportion of Ih- cells than Ih+ cells exhibited potentiation (Ih+: 80% potentiated, Ih-: 38% potentiated). These data suggest that local

GABA neurons are capable of exhibiting LTP_{GABA} on most cells in the VTA but that local inputs onto certain subtypes of Ih- neurons may not express LTP_{GABA}In contrast, inhibitory synapses arising from the RMTg do not express LTP in either Ih+ or Ih- cells (LTP magnitude, Ih+: 107 ±15% of baseline, n=7; Ih-: 90 ±7% of baseline, n=4). In addition, we investigated whether dopamine neurons projecting to specific target regions were capable of expressing LTP_{GABA}. We find that NAc-projecting dopamine neurons exhibit SNAP-induced LTP_{GABA} (LTP magnitude, 125 ±7% of baseline, n=6). Taken together, our studies indicate that subcircuits within the VTA differ in their expression of LTP at inhibitorysynapses, allowing differential modulation of these inputs by experience.

Disclosures: **K. Barcomb:** None. **A.M. Polter:** None. **A.C. Tsuda:** None. **J.A. Kauer:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

Location: Halls B-H

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Program#/Poster#: 456.01/JJJ43

Topic: G.06. Post-traumatic Stress Disorder

Title: Sex-specific phenotypes in a rat model of post-traumatic stress disorder (PTSD)

Authors: ***A. POOLEY**, A. J. ROBISON, M. S. MAZEI-ROBISON, A. L. EAGLE, S. M. BREEDLOVE, C. L. JORDAN;
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Abstract: Men and women respond differently to traumatic stress, but the neurobiological basis for this is not understood. Diagnoses and treatment of post-traumatic stress disorder (PTSD) would likely benefit by understanding these sex differences. Women are twice as likely as men to develop symptoms of PTSD following a traumatic experience. They are also affected longer, and tend to re-experience trauma more than men through flashbacks, nightmares, and intrusive memories. Clinical studies indicate that women with PTSD are more likely to show an internalizing phenotype (associated with comorbid anxiety and depression) whereas men are more likely to show an externalizing phenotype (associated with comorbid aggression, substance abuse and impulsivity). Our data begin to shed light on the neurobiological reasons why men and women respond differently to traumatic stress. The single prolonged stress (SPS) paradigm is a well-validated rat model for PTSD, recapitulating clinical symptoms of the disorder in male rats—enhanced startle, dysregulation of the hormonal stress response, and changes in corticolimbic activation. However, we are the first to compare the effects of SPS in male and female rats at the behavioral, physiological, and cellular levels. We found the acoustic startle response to be exaggerated in males following SPS, as expected, but SPS did not affect the

startle response of females. However, based on the dexamethasone suppression test (DST) and neuronal activation in the prefrontal cortex, females are in fact affected by SPS. We are the first to show a behavioral effect of SPS in females where females, but not males, showed a depressive-like anhedonic effect on sucrose intake following SPS. Given this striking sex difference, we then sought to determine whether normal sex differences in adult gonadal hormones mediate these responses. We find that removing testicular hormones eliminates the difference in the acoustic startle response between stress-exposed and control males, reflecting a loss in the anxiolytic effect of endogenous androgens in males. On the other hand, the effect of SPS on sucrose intake in females may be independent of ovarian hormones. Taken together, these data begin to uncover novel neurobiological mechanisms underlying sex-specific responses to trauma that may pave the way for the development of new sex-specific diagnostic tools and treatments for men and women with PTSD.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.02/JJJ44

Topic: G.06. Post-traumatic Stress Disorder

Support: DARPA

NIH

SBIR

Title: Vagus nerve stimulation reverses extinction impairments and alters PTSD symptoms in the SPS animal model

Authors: *L. J. NOBLE, I. J. GONZALEZ, V. B. MERUVA, A. K. HUTCHINSON, T.-A. DAM, S. K. THOMAS, E. MEYERS, M. P. KILGARD, C. K. MCINTYRE;
Univ. of Texas At Dallas, Dallas, TX

Abstract: Posttraumatic stress disorder (PTSD) can develop following a traumatic event. Symptoms of PTSD include hypervigilance, avoidance, and increased anxiety. Exposure therapy is a form of cognitive behavioral therapy that is commonly used to treat these symptoms. During exposure therapy, patients are repeatedly exposed to cues that remind them of the trauma until they learn healthier responses to those cues. However, PTSD patients show impairments in

extinction learning, which may increase nonresponse rates and dropout rates. Adjuncts to exposure therapy could be utilized to increase the effectiveness by promoting successful extinction learning, this could lead to decreases in nonresponse and dropout rates. Vagus nerve stimulation (VNS) is an FDA-approved treatment for the prevention of seizures. VNS shows promise as an adjunct for exposure therapy because previous research indicates that it enhances memory consolidation in rats and in humans, and we recently found that pairing VNS with unreinforced exposures to a conditioned stimulus can enhance extinction learning and protect against relapse in the single prolonged stressor (SPS) rat model of PTSD that shows resistance to extinction. The current studies are designed to test the hypothesis that extinction impairments contribute to general symptoms of PTSD and, therefore, treatments that promote successful extinction should produce benefits that reach beyond the cue-induced fear. SPS and control rats were fear conditioned for two days, then given daily extinction training with VNS or sham stimulation. One week, and six weeks later, rats were given tests of hypervigilance (startle), avoidance (conditioned place avoidance), and general anxiety (elevated plus maze). We hypothesize that reversing extinction impairments in SPS-treated animals with VNS will decrease anxiety, avoidance, and hypervigilance. Results indicate that VNS pairing with extinction training brought fear expression in SPS-treated rats to control levels, and increased time spent in the open arms of an elevated plus maze (versus sham-treated SPS rats) one week after extinction training. These results suggest that VNS enhancement of extinction also provides benefits for additional PTSD symptoms seen in the SPS model.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.03/JJJ45

Topic: G.06. Post-traumatic Stress Disorder

Support: NARSAD Brain and Behavior Foundation Grant 22683

NIH Grant MH105400

Title: A preclinical mouse model of traumatic memory storage- implications for PTSD

Authors: *S. SILLIVAN, N. JOSEPH, C. MILLER;
The Scripps Res. Institute-Florida, Jupiter, FL

Abstract: How does the brain stably maintain memories for a lifetime? Understanding how the brain maintains a memory has significant implications for disorders perpetuated by lasting, pathogenic memories, such as post-traumatic stress disorder (PTSD). Despite the clinical importance of this memory process, mechanisms of long-lasting ("remote") memory are understudied and remarkably elusive. PTSD is precipitated by an individual experiencing or witnessing a traumatic event that later leads to substantial dysfunction in fear processing, including hyperactivation of the amygdala (AMY) upon exposure to fearful stimuli and generalization of fearful responses to nonfearful stimuli. The AMY is the brain's emotional memory center and, unlike hippocampus-dependent memories, which shift to the cortex over time, AMY-dependent memories continue to rely on the AMY weeks after learning. In rodents, a "normal" fear memory can be converted to a traumatic memory by pre-exposure to stress. Subsequent fear conditioning (FC) results in a fear memory that displays greater resistance to extinction than one formed in the absence of prior stress (stress enhanced fear learning; SEFL). We have fine-tuned an SEFL paradigm that combines restraint stress with FC to precipitate traumatic memories in male mice. Characterization of this SEFL paradigm revealed strong face validity to PTSD: (1) extinction resistance, (2) generalization of fear (3) differential vulnerability (stress-resilient and -susceptible phenotypes arise), (4) persistence of elevated fear for more than 30 days and (5) a dose-dependent response of the stressor. Given that the prevalence of PTSD is nearly twice as high in the female population, we also performed the SEFL paradigm in female mice. Gender specific differences in fear extinction and retention were observed in female mice compared to their male counterparts, suggesting that female mice are more sensitive to the stress component of this paradigm. To identify molecular components that contribute to traumatic memory storage, RNA sequencing was performed on AMY tissue from SEFL male mice that did not undergo extinction, 30 days after FC shock training. Bioinformatic analysis of genes changed in 'PTSD-like', stress-susceptible mice indicated that this paradigm recruits major neurotransmitter systems and pathways involved in critical neuronal processes, such as CREB signaling, cAMP-mediated signaling, corticotropin releasing hormone signaling, and protein kinase A signaling. This preclinical PTSD model represents a valuable tool for studying long-lasting memory mechanisms, which has significant therapeutic implications for those suffering from PTSD.

Disclosures: S. Sillivan: None. **N. Joseph:** None. **C. Miller:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.04/JJJ46

Topic: G.06. Post-traumatic Stress Disorder

Title: PTSD-like behavioral profile of mice with full 5-HT2C receptor editing: response to paroxetine treatment.

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Abstract: Post-traumatic stress disorder (PTSD) is a stress-related disorder caused by exposure to a psychological trauma. No pharmacological treatment demonstrated a wide efficacy and there are only few preclinical PTSD models that meet face and predictive validity. PTSD is characterized by a deregulation of fear responses, including fear extinction deficits and generalization. Serotonin 2C receptor (5-HT2CR) editing impacts anxiety-like behaviors, and we recently demonstrated in the fear conditioning paradigm, that mice expressing only the fully edited 5-HT2CR isoform (VGV mice) display increased innate fear behaviors as well as fear extinction deficits and context generalization. Interestingly, 5-HT2CR inactivation has been shown to alter the expression of the brain-derived neurotrophic factor BDNF, known to regulate memory, and proposed to play a central role in PTSD. In this context, VGV mice were characterized as an animal model for PTSD. Wild-type (WT) and VGV adult C57Bl/6 mice were used to assess fear-related behaviors (ultrasound test and fear conditioning), memory and flexibility (Barnes maze). WT and VGV mice also received either paroxetine (delivered in drinking water; ~5.5 mg/kg/day) or tap water during 28 days. Then cue fear conditioning (6 x 2.5 kHz CS, 0.5 mA foot-shocks) was conducted to evaluate fear extinction (40 x CS alone). Fear behavior was also assessed in reaction to an innately aversive ultrasonic stimulus. Brains were dissected for qRT-PCR analyses of *Bdnf* gene expression. Data were analyzed using the Student's t-test and the two-way ANOVA. Chronic paroxetine treatment in VGV mice reduced freezing in both fear conditioning and ultrasound-induced fear paradigms and partially reversed contextual fear generalization. However, as previously reported, chronic paroxetine impaired rather than improved the extinction process in WT mice. Furthermore, this treatment normalized *Bdnf* mRNA dysregulations in the hippocampus and the amygdala of VGV mice. Interestingly, VGV mice displayed no alteration of spatial learning and no impairment of cognitive flexibility. Our data showed that, while presenting no general cognitive impairment, VGV mice displayed severe fear memory dysregulations, similar to those of PTSD patients, which could be partially prevented by paroxetine. Our results also provided further evidence that BDNF may be a factor underlying PTSD-like behaviors. VGV mice are thus a relevant animal model to design new pharmacological strategies for PTSD.

Disclosures: **M. Règue:** None. **C. Poilbout:** None. **L. Lanfumey:** None. **R. Mongeau:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant R01DA027688

NIH Grant P50MH103222

Title: Alternations in fear behavior following acute stress in adrenalectomized rats: Involvement of kynurenic acid and implications for PTSD

Authors: *D. J. BUCCI[1], N. E. DEANGELI[1], K. S. HERRINGTON[1], H.-Q. WU[2], R. SCHWARCZ[2];
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Abstract: Kynurenic acid (KYNA) is a metabolite of tryptophan degradation and acts as an antagonist of both NMDA glutamate and alpha-7 nicotinic acetylcholine receptors. Prior work has shown that endogenous KYNA modulates dopamine levels in the rodent brain. For example, the well-established and purportedly adaptive increase in dopamine levels in the medial prefrontal cortex following an acute restraint stress is abolished in rats after adrenalectomy (ADX), and this effect is secondary to an abnormal increase in extracellular KYNA. As the response to stress is abnormal in several major psychiatric diseases, we hypothesized that there may be a link between stress-induced changes in KYNA and behavior, especially in pathological situations. Here we tested the behavioral relevance of this stress-induced increase in KYNA by training ADX rats (or sham-operated controls) in a fear discrimination procedure. One set of rats in each group was exposed to a 2-hour restraint stress while the others remained in the home cage (ie, a 2X2 experimental design). Immediately thereafter, all rats underwent fear conditioning in which one auditory conditioned stimulus (CS+) was paired with mild foot shock and a second auditory cue (CS-) was not paired with shock (three trials of each, intermixed over a 6-min session). Forty-eight hours later, rats were tested for conditioned fear to each cue by re-exposing them to the auditory stimuli in a novel context without any shocks delivered. Sham-operated control rats exhibited more conditioned fear (freezing behavior) to the CS+ than the CS-, regardless of whether they underwent restraint stress. Similarly, ADX rats that were not restrained exhibited more fear to the CS+ than the CS-. In contrast, ADX rats exposed to 2 hours of restraint stress exhibited high levels of fear to both stimuli, reminiscent of impairments in fear learning exhibited by persons with post-traumatic stress disorder (PTSD). Ongoing studies are testing the specific role of KYNA in mediating these behavioral effects.

Disclosures: **D.J. Bucci:** None. **N.E. DeAngeli:** None. **K.S. Herrington:** None. **H. Wu:** None. **R. Schwarcz:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.06/JJJ48

Topic: G.06. Post-traumatic Stress Disorder

Title: Influence of estrous stage on the behavioral response of female rats to a predator-based psychosocial stress model of PTSD

Authors: ***E. D. EISENMANN**, R. M. ROSE, M. E. FRY, B. L. JOHNSON, M. R. HUNTLEY, M. E. HEIKKILA, B. A. KOHLS, P. R. ZOLADZ;
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Abstract: Although females are more likely to develop post-traumatic stress disorder (PTSD), a female animal model of PTSD is still non-existent. Here, we have examined the effects of an animal model of PTSD, previously validated in male rats, on female rats and how estrous stage might influence any effects. Female Sprague-Dawley rats were exposed to psychosocial stress or control conditions for 31 days; vaginal smears were collected on days 1, 11 and 32 to determine estrous stage. Stressed rats were given two cat exposures, separated by 10 days, and subjected to daily social instability throughout the paradigm. Control rats were handled daily. Rats were tested on the elevated plus maze (EPM) on day 32 and in an open field on day 33. Results indicated that estrous stage during testing interacted with stress to affect behavior. Specifically, stressed females in estrus spent less time in the open arms of the EPM than controls. Additionally, stressed females in estrus or diestrus were less mobile and made fewer rearing episodes in the open field than controls. This work provides preliminary evidence for an interaction between female hormones and chronic stress and could be useful for understanding susceptibility factors for PTSD in females.

Disclosures: **E.D. Eisenmann:** None. **R.M. Rose:** None. **M.E. Fry:** None. **B.L. Johnson:** None. **M.R. Huntley:** None. **M.E. Heikkila:** None. **B.A. Kohls:** None. **P.R. Zoladz:** None.

Poster

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Program#/Poster#: 456.07/JJJ49

Topic: G.06. Post-traumatic Stress Disorder

Title: Clonidine prevents the anxiogenic, but not cardiovascular, consequences of a predator-based psychosocial stress model of PTSD

Authors: *M. E. FRY[1], E. D. EISENMANN[1], R. M. ROSE[1], B. L. JOHNSON[1], M. R. HUNTLEY[1], M. E. HEIKKILA[1], K. L. ROBINSON[1], B. R. RORABAUGH[2], P. R. ZOLADZ[1]; [1]Psychology, Sociology, & Criminal Justice, [2]Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH

Abstract: Individuals with PTSD are at increased risk for cardiovascular disease. We previously reported that a predator-based psychosocial stress model of PTSD led to greater myocardial sensitivity to ischemic injury. Here, we examined whether chronic administration of the noradrenergic antagonist clonidine would prevent such effects. Male Sprague-Dawley rats were exposed to psychosocial stress or control conditions for 31 days. Stressed rats were given two cat exposures, separated by 10 days, and subjected to daily social instability throughout the paradigm. Control rats were handled daily. Beginning on day 2, rats received daily injections of 0.05 mg/kg clonidine or vehicle (saline), continuing through day 32. Rats were tested on the elevated plus maze (EPM) on day 32, and on day 33, rat hearts were isolated and subjected to 20 min ischemia and 2 hr reperfusion on a Langendorff isolated heart system. Consistent with previous work, clonidine blocked the development of anxiety-like behavior in stressed animals. However, clonidine was ineffective at preventing the stress-induced increase of myocardial sensitivity to ischemic injury. Stressed rats, overall, exhibited larger infarct sizes and reduced post-ischemic recovery of contractile function relative to controls. These findings suggest a potential dissociation between the anxiogenic and cardiovascular effects of this chronic psychosocial stress paradigm.

Disclosures: **M.E. Fry:** None. **E.D. Eisenmann:** None. **R.M. Rose:** None. **B.L. Johnson:** None. **M.R. Huntley:** None. **M.E. Heikkila:** None. **K.L. Robinson:** None. **B.R. Rorabaugh:** None. **P.R. Zoladz:** None.

Poster

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Program#/Poster#: 456.08/JJJ50

Topic: G.06. Post-traumatic Stress Disorder

Title: Decreased voluntary ethanol consumption in a predator-based psychosocial stress model of PTSD

Authors: *R. M. ROSE, E. D. EISENMANN, B. L. JOHNSON, M. E. FRY, M. E. HEIKKILA, M. R. HUNTLEY, P. R. ZOLADZ;
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Abstract: Individuals with post-traumatic stress disorder (PTSD) exhibit high rates of substance abuse, which may be due to self-medication. Here, we have examined whether a well-established animal model of PTSD would influence voluntary ethanol intake in rats. Male Sprague-Dawley rats were exposed to psychosocial stress or control conditions for 31 days. Stressed rats were given two cat exposures, separated by 10 days, and subjected to daily social instability throughout the paradigm. Control rats were handled daily. Beginning on day 32, rats were given access to either ethanol (10% EtOH + 1% sucrose) or water (1% sucrose) in 12-hr cycles (1930-0730 every night) using a two bottle, free choice paradigm for 21 days. Results revealed that stressed rats consumed less ethanol than control rats throughout the 21 days of ethanol exposure. It is possible that stressed rats consumed less ethanol because they exhibited a greater aversion to the novel stimulus, or the chronic stress paradigm altered drug sensitivity such that the stressed rats required less ethanol to induce the desired effect. Follow-up studies are currently in progress to delineate such possibilities.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: IRP/NIAAA/NIH

R01MH094489 (PDS,GIE)

Title: Adolescent trauma results in distinct behavioral covariates and neural activation patterns in habenula associated brain regions

Authors: *G. I. ELMER[1], J. R. SCHANK[2], J. MITCHELL[3], R. DAMAZDIC[2], C. L. MAYO[1], D. BRADY[1], A. PINCUS[2], C. KING[2], M. HEILIG[2], J. D. TAPOCIK[2];
[1]Psychiatry, Maryland Psychiatric Res. Ctr., Baltimore, MD; [2]Lab. of Clin. and Translational Studies,, NIAAA, NIH, Bethesda, MD; [3]Psychology, Colby, Waterville, ME

Abstract: The consequences of trauma, especially early life trauma, infiltrate psychiatric illness across a range of diagnoses including major depressive disorder, generalized anxiety and substance abuse making trauma exposure a highly penetrable, life-changing event. Unfortunately, the neurobiology underlying the consequences of trauma exposure is poorly understood and effective models and treatment interventions are slow in development.
The majority of currently used animal models rely on acute physical stress or exposure to predator scent to mimic trauma. While these paradigms produce stress-related phenotypes, physical and odor-induced stress are not directly life-threatening and may not induce the neural events necessary for a robust animal model. We have developed an animal model that directly exposes the subject in close contact with a live predator (LPE) yet prevents physical harm. In the model, male Wistar rats are exposed a rat snake (60-100cm long) for 10 min at post-natal days (PND) 31, 46 and 61 by placing rats in a perforated restrainer tube within a larger arena that holds the live snake. Twelve days following the last snake exposure rats are assessed in a battery of behavioral tests. In the current experiment, rats were perfused and tissue prepared for cFOS analysis in six brain regions.
LPE produces robust behavioral and neurobiological consequences. Exposed rats had significantly blunted corticosterone response following the third snake exposure, decreased exploratory behavior and increased defecation in open field, decreased open arm entries in elevated plus maze, decreased saccharin preference, increased conditioned fear and increased rates of learned helplessness. cFOS analysis revealed significant increased expression in the lateral habenula (LHb) and dorsal raphe following repeated LPE. Analysis of a correlation matrix across brain regions revealed a change in strength of regional associations following LPE as measured by cFOS covariance. In particular, LPE resulted in a significant correlation between lateral LHb and RMTg cFOS expression that survived multiple comparisons correction. In addition, there was a strong correlation (short of multiple corrections threshold) between the nucleus incertus and medial LHb and dorsal raphe brain regions.
Further investigation is required to determine if the association between regions is causal or merely reflects co-activation. The combination of roles hypothesized to be involved in LHb and RMTg-associated circuitry place this network in an ideal situation to mediate core symptom constructs altered by trauma exposure that cross diagnostic borders.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIDA Grant DA031900

Title: Susceptibility to traumatic stress predicts elevations in cocaine self-administration and the dopaminergic response to cocaine.

Authors: *Z. D. BRODNIK[1], M. CLARK[2], K. KORNSEY[2], R. A. ESPAÑA, 19129[2];
[1]Drexel Univ., Philadelphia, PA; [2]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Patients with post-traumatic stress disorder have a heightened vulnerability to developing substance use disorders. Despite clear clinical evidence for this vulnerability, preclinical studies of prior stress experience on cocaine self-administration behavior have produced inconsistent results. We used the predator order stress model of post-traumatic stress disorder with segregation of subjects as susceptible or resilient based on elevated plus maze behavior and context avoidance. We then determined behavioral and neurochemical differences across susceptible, resilient, and control populations using cocaine self-administration, in vivo microdialysis, and ex vivo fast scan cyclic voltammetry. Susceptible subjects showed an increased propensity to self-administer cocaine, which corresponded with increased basal levels of nucleus accumbens dopamine, and increased dopamine responses to cocaine. Resilient subjects did not show changes in cocaine self-administration or in vivo dopamine signaling. Nonetheless, we found increases in both the dopamine release-promoting effects of cocaine and dopamine autoreceptor sensitivity ex vivo. Our results suggest that traumatic stress results in alterations to dopamine systems that drive an increase in the propensity for susceptible subjects to self-administer cocaine. In contrast, traumatic stress results in both active and passive forms of resilience that function to prevent gross changes in cocaine self-administration for resilient subjects.

Disclosures: **Z.D. Brodnik:** A. Employment/Salary (full or part-time): Drexel University College of Medicine. **M. Clark:** None. **K. Kornsey:** None. **R.A. España:** A. Employment/Salary (full or part-time): Drexel University College of Medicine.

Poster

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant R01MH098003

NIH Grant R01NS085200

Title: Awake resting-state fMRI in a post-traumatic stress disorder rat model

Authors: *P. D. PEREZ, D. DOPFEL, L. ANTINORI, J. RUDDY, N. ZHANG;
Biomed. Engin., Pennsylvania State Univ., University Park, PA

Abstract: A stress response occurs when an individual perceives a threat to its safety that can potentially exceed its coping resources. Psychophysiological responses are directed towards adaptation. In the event of maladaptation, strong traumatic stimuli may produce chronic stress disorders that can develop into excessive anxiety, phobias and post-traumatic stress disorder (PTSD). The mechanisms of PTSD are poorly understood since most studies in humans focused on populations that had already been exposed to a wide variety of different traumatic experiences such as war veterans and victims of accidents or crime. Using animal models allows for investigating the development of PTSD at different points in time. This includes the stage before the exposure to stress which is very difficult to observe in humans. We present a rat model of PTSD where we exposed rats to a single-episode traumatic event in the form of predator odor. Our study investigates the neuroplasticity changes at the circuit level to a single life threatening situation using awake resting-state functional magnetic resonance imaging (rsfMRI). By using awake imaging, we can avoid the confounding factors that usually accompany the use of anesthetics. Behavior techniques are also used to evaluate the efficacy of stress trauma. Furthermore, we explore how fear conditioning and extinction mechanisms are modulated by the traumatic event. These learning processes and their dysregulation are believed to be at the core of chronic stress disorders. We present a longitudinal group of experiments with multiple goals. First, the impact of single-episode trauma and the onset of chronic stress disorders are studied. We then investigate their effects in fear conditioning and later fear extinction processes. Our study allows us to look for vulnerability markers to PTSD at the circuit level with non-invasive techniques thus segregating the exposed rats in resilient and vulnerable populations. The understanding and detection of vulnerability to PTSD is of great importance in the prevention and treatment, opening the way to extending this knowledge to humans.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: NSC 102-2410-H-431-005-MY3

Title: Do opiate and hypothalamus-pituitary-adrenal gland systems affect fear, depression, and movement behaviors for posttraumatic stress disorder in rats?

Authors: *C. CHIU[1], A. C. W. HUANG[2];
[1]Dept. of psychology, Fo Guang Univ., Taiwan (R.O.C.), Taiwan; [2]Psychology, Fo Guang Univ., Yilan County, Taiwan

Abstract: Posttraumatic stress disorder (PTSD) is a severe anxiety disorder. PTSD patients often suffer from fear major symptom and some comorbidity including depression and anxiety behaviors. However, whether the opiate and hypothalamus-pituitary-adrenal gland (HPA) systems influence fear, depression, anxiety, and movement behaviors remains unclear. The aim of the present study addresses this issue. All of rats receive a saline or 10 mg/kg morphine subcutaneously injection 30 min prior to a footshock treatment. Then, rats were placed in the footshock box for 2 min and receive a footshock (3 mA, 10s) to produce a severe trauma. Next day, rats were given into 0.5 mg/kg dexamethasone (DEX) or its vehicle once a day for three days. During this period of time, situational reminder was given. With no footshock, rats were placed in the footshock box for 2 min. Fear behavior was measured. After that, rats were respectively tested depression, and movement behaviors in the forced swimming and open field tests.
The present data showed that morphine or DEX injections might decrease freezing time and significantly reduced freezing time compared with the Saline/VEH group, indicating morphine or DEX might decrease fear behavior. The combination of morphine and DEX administrations exhibited the lower freezing time than the single morphine and DEX groups. The synergism of morphine and DEX showed a highest value of swimming time, indicating the addition of morphine and DEX can effectively reduce depression. However, either morphine or DEX cannot affect movement and moreover, the addition of morphine and DEX cannot influence movement. The present data can provide clinical implications and thereby it can give us knowledge to understand how the opiate system and HPA axis involve in fear, depression, and motor functions to PTSD patients.
Keywords: posttraumatic stress disorder, opiate system, hypothalamus-pituitary-adrenal gland axis, fear, depression, motor, rat

Disclosures: C. Chiu: None. **A.C.W. Huang:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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NIH grant T32 AA007474

VA Medical Research

Title: Oxytocin attenuates stress-induced reinstatement of alcohol seeking in mice with a history of trauma

Authors: *C. KING, W. C. GRIFFIN, J. F. MCGINTY, H. C. BECKER;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Exposure to chronic stress can lead to serve psychiatric issues including post-traumatic stress disorder (PTSD), anxiety and depression, many of which are often complicated by the presence of concurrent substance or alcohol use disorder. An amassed body of evidence has recently emerged to support a role for the endogenous neuropeptide oxytocin (OXT) system in both drug-seeking and stress-related behaviors. Previous research in our laboratory has demonstrated that OXT reduces ethanol in a variety of drinking paradigms. The present study was designed to extend these findings by examining the effects of OXT on stress-induced reinstatement of alcohol seeking in mice with and without a history of repeated predator odor exposure stress. Adult C57BL/6J mice (n=48) were trained to acquire stable rates of lever responding under a fixed ratio (FR)-4 schedule for 12% ethanol in 20-min sessions. Once active lever response rates and ethanol intake had stabilized (<15% variability across 3 consecutive days), mice were injected with yohimbine (2mg/kg) or saline 15 minutes prior to exposure to predator odor (2,3,5-Trimethyl-3-thiazoline; TMT-PE) or saline (CTRL), for 5 consecutive days. Next the mice were returned to active self-administration to re-establish baseline levels of responding, followed by an extinction phase (14 days) in which no cues or alcohol reward was present. At the conclusion of the extinction phase, mice were injected with OXT (1mg/kg) or saline 30 minutes prior to 15 minute TMT exposure. Reinstatement session was immediately following TMT exposure. Results indicated mice with a history of TMT exposure showed an exacerbated stress-induced reinstatement compared to mice with history of saline exposure ($p<0.001$). Further, OXT treatment attenuated reinstatement in the TMT-PE group ($p<0.0001$).

These results implicate a role for oxytocin in decreasing stress-induced reinstatement of alcohol seeking. Additional studies are underway to examine the effects of OXT treatment of neurobiological markers of stress, as well as the mechanism through which oxytocin may be modulating stress-induced reinstatement, particularly in animals with a history of trauma. Supported by DoD/US Army Institute for Molecular Neuroscience 803-94, NIH grants P50 AA10761and U01 AA014095, and T32 AA007474, and VA Medical Research.

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Poster

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant 1R01MH098003-01A1

Title: Effects of acute immobilization stress on brain network functional connectivity and its use as a PTSD model

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Abstract: Post-traumatic stress disorder development is difficult to study in humans. It is difficult to screen patients prior to trauma and the stress response immediately after trauma is typically not studied in people. This creates a gap in our understanding of the disorder that can be filled by animal models. Work by Mitra et al. analyzed the effect of stress duration on behavioral and cellular changes in acute immobilization stress paradigm in rats. They found that acute immobilization stress (AIS) leads to morphological changes and behavioral manifestations reminiscent of anxiety. The increase in spine formation in the basolateral amygdala and behavioral manifestations of anxiety caused by a single event stressor suggest the relevance of this paradigm as a PTSD model. To explore this model further male Long-Evan rats were subjected to AIS. To further validate this model, behavioral cut-off criteria were used to determine the assignment of vulnerable or susceptible rats within the stressed group. The prevalence of vulnerable rats was found to be similar to prevalence of PTSD in people that have experienced a traumatic event. Furthermore, work by Roa et al. suggests that glucocorticoids protect against behavioral and cellular effects of stress. To better understand the role of glucocorticoids in the stress response and in a model of PTSD, serum corticosterone levels were

measured at multiple time points throughout the experiment. Baseline, stress response and post stress levels were analyzed in relation to the susceptibility of the animals and behavioral manifestations of anxiety. The separation of vulnerable and susceptible animals allows for a further study of neurological differences between individuals before and after the occurrence of the traumatic event using awake rodent resting state functional magnetic resonance imaging (rs-fMRI). rs-fMRI suggests a variation in functional connectivity patterns between the vulnerable and susceptible animals. This is novel from a similar imaging study in our lab, due to a stressor of a different modality. In fact, combining these two studies data sets will allow the study of changes in stress response caused by a variation in the stressor and a further validation of the changes of network connectivity due to stress that may form the basis of future rs-fMRI pre-diagnostic/diagnostic screening of populations (e.g. soldiers) for PTSD susceptibility. Information gained from the monitoring of glucocorticoid levels allows for a separate diagnostic measure of susceptibility and may validate glucocorticoid supplementation as a therapeutic option for those that are at an acute risk of trauma or have experienced a traumatic event.

Disclosures: **D. Dopfel:** None. **N. Zhang:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH–NIGMS 8P20GM103471-09

State of Nebraska LB692

Title: Role of alpha2-adrenergic receptors in modulating post-traumatic stress disorder-like behaviors in a novel fearful DxH congenic recombinant inbred mouse strain with a DBA/2J background

Authors: R. WICKRAMASEKARA, Y. FARHAT, S. AKKOSEOGLU, *D. M. YILMAZER-HANKE;
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Abstract: Post-traumatic stress disorder (PTSD) results from exposure to highly stressful and aversive traumatic events that lead to re-experiencing of the trauma, intrusive thoughts and flashbacks, which are often modeled in the fear-conditioning paradigm, as well as to hyperarousal and anhedonia. These symptoms can be accompanied by elevated baseline

noradrenaline levels and provocation of flashbacks and panic attacks following delivery of the alpha2-adrenergic receptor antagonist yohimbine (Yoh). We have developed a novel fearful and stress-prone C3H-like recombinant inbred (C3HLRI) mouse strain by backcrossing C3H/HeJ mice selected for a high fear-sensitized acoustic startle response (FSS) onto DBA/2J mice with a low FSS. Compared to control DBA/2J mice, the congenic C3H-like recombinant inbred (C3HLRI) mice show increased startle reactivity, depressive-like behavior in the forced swim test, enhanced basal tissue noradrenaline levels, and a poor noradrenergic response to stress as seen in PTSD patients. In the present study, we applied Yoh to test the hypothesis that fearful/stress-prone C3HLRI mice are a model for PTSD using the open field and auditory fear-conditioning tests. The alpha2-adrenergic receptor agonist clonidine (Clon) was used to reverse Yoh effects. Six week old C3HLRI and DBA/2J mice received i.p. injections of Yoh (2.5 and 5.0 mg/kg), Clon (0.06, 0.08 and 0.1 mg/kg) or saline 30 min before behavioral testing. Yoh and Clon both reduced locomotion in a dose dependent manner in the open field test. Anxiety-like measures assessed in the open field indicated increased anxiety levels with Yoh and reduced anxiety levels with Clon in C3HLRI mice but not control DBA/2J mice. Anxiolytic effects of Clon were blocked by Yoh when the mice were administered the antagonist Yoh shortly before Clon delivery. In fear-conditioning, Yoh induced a fear extinction deficit in both strains, however, overall freezing levels were higher in C3HLRI mice. Plasma corticosterone levels were increased 30 minutes after Clon injection in both strains, although no significant differences were observed between the two strains. In summary, Yoh provoked increased anxiety and fear levels in the open field and fear-conditioning tests in C3HLRI but not DBA/2J mice. Along with previous data on higher conditioned fear levels, depressive-like behavior, enhanced stress reactivity, and noradrenergic changes in C3HLRI mice compared to DBA/2J mice, the present findings support the hypothesis that C3HLRI mice may serve as a PTSD model.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Support: IRC Grant 1-19129

VT Sigma Xi Research Award 4-44130

Title: Single prolonged stress-induced deficits in fear extinction recall are affected by exposure to functional modulators of astrocytic glutamate transport

Authors: T. S. COTRONE[1], *B. S. JORTNER[2], M. F. EHRICH[1], B. G. KLEIN[1];
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Abstract: Persistence of intrusive fear memories in Post-Traumatic Stress Disorder (PTSD) may be more attributable to a trauma-induced, pre-frontal cortex (PFC) -mediated dysfunction of fear extinction memory compared with an amygdala-mediated dysfunction of fear memory formation. There is also evidence that stimulating extrasynaptic NMDA glutamate receptors impairs long-term potentiation at glutamatergic synapses. We are exploring the hypothesis that a PTSD-related situational trauma impairs astrocyte-mediated glutamate reuptake in PFC, leading to increased extrasynaptic NMDA receptor activation, resulting in impairment of PFC-mediated fear extinction memory. We employed the Single Prolonged Stress (SPS) protocol as a rat model of PTSD in a 2 trauma (SPS; No SPS) x 3 drug (ceftriaxone; dihydrokainate; saline) factorial design. Ceftriaxone (CEF) was used to increase glutamate astrocytic transporter (GLT-1) concentration, dihydrokainate (DHK) was used to block GLT-1, and saline was used as a sham treatment. 7 days after SPS or No SPS, all rats were exposed to a light-cued fear conditioning paradigm, followed 24 hrs later by an extinction session, followed 24 hrs later by an identical extinction session to assess fear extinction memory. Rats were sacrificed after the second extinction session and the hippocampus, amygdala, and prefrontal cortex were removed for Western blot of glucocorticoid receptor (GR) and GLT-1. For saline-treated rats, SPS exposure resulted in significantly poorer fear extinction recall compared to the No SPS group. Treatment with CEF, intended to increase GLT-1-mediated uptake of glutamate and reduce extrasynaptic NMDA receptor stimulation in PFC, eliminated this SPS-induced impairment in fear extinction recall. However, DHK treatment, intended to block GLT-1-mediated uptake of glutamate and increase extrasynaptic NMDA receptor stimulation in PFC, also eliminated this SPS-induced impairment. In addition to these results for fear extinction recall, analyses revealed effective fear acquisition and fear extinction in all experimental groups, with magnitudes unaffected by trauma condition or drug treatment. Western blot analysis confirmed, in our SPS rats, the previously-reported increase in hippocampal GR. It also confirmed, in No SPS rats, that our CEF treatment was increasing GLT-1 protein concentration equally in PFC and amygdala. These results suggest that modulation of astrocytic glutamate reuptake can affect fear extinction recall deficits in rats exposed to a situational trauma analog of PTSD, but the specific mechanisms are not clear at this time. Support: IRC Grant 1-19129 and VT Sigma Xi Research Award 4-44130.

Disclosures: **T.S. Cotrone:** None. **B.S. Jortner:** None. **M.F. Ehrich:** None. **B.G. Klein:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.17/JJJ59

Topic: G.06. Post-traumatic Stress Disorder

Title: Animal models of post traumatic stress disorder: behavioral characterization and pharmacological validation

Authors: *I. MORGANSTERN, Q. CHANG, A. CHOO, L. THIEDE, K. HOMA, E. SABATH, W. ALVINS, J. SUTPHEN, M. LANG, S. DAVIS, T. HANANIA;
Behavioral Pharmacol., Psychogenics, Tarrytown, NY

Abstract: Posttraumatic stress disorder (PTSD) is a common anxiety disorder characterized by hyper-arousal, disturbing flashbacks and numbing or avoidance of memories of a traumatic event or experience (DSM V, 2013). In order to fill the current gap in PTSD treatment research and increase our understanding of the underlying neurobiological and pathophysiological mechanisms, the development and validation of robust preclinical models with high face and construct validity is absolutely necessary. The current series of studies present findings from two animal models of PTSD, namely the mouse chronic social defeat (CSDS) model (Berton et al., 2006; Golden et al., 2011) recently developed in collaboration with Dr. Eric Nestler and also the fear-conditioned, Wistar- Kyoto (WKY) rat model (DaSilva et al., 2011; 2013). Specifically, our data with the CSDS model suggests that animals most susceptible to social stress after going through the 10-day social defeat paradigm exhibit increased social avoidance behavior, anhedonia and potentiated fear responding to sound cues. Most interesting are the findings with our proprietary algorithm-based behavioral SmartCube ® system, which measures whole animal behavior. These analyses demonstrated very unique and specific behaviors related to anxiety and fear of the susceptible versus control mice that progressed over time and were evident as long as 6 weeks after the social defeat stress. In a separate series of experiments, we characterized the extinction profile of fear-conditioned WKY rats (0.6mA shock, 6 shocks over 8 min). The findings suggest that compared to their outbred counterparts (Wistar), this stress-sensitive rat strain (WKY) exhibits deficits in fear extinction that are pronounced and long lasting. Pharmacological data with acute or intermittent ketamine treatment as well as compounds targeting more traditional mechanisms related to 5-HT or adrenergic signaling will also be presented for these models. Collectively, the data gathered from these two preclinical models offers a dynamic portfolio of PTSD-relevant behavioral profiles (anxiety, social avoidance, abnormal fear regulation) that can potentially be utilized as a tool to screen novel drug compounds. Our pharmacological evidence demonstrates the predictive validity of these models and further supports the use of CSDS mouse model and WKY rat model in future PTSD drug testing.

Disclosures: **I. Morganstern:** None. **Q. Chang:** None. **A. Choo:** None. **L. Thiede:** None. **K. Homa:** None. **E. Sabath:** None. **W. Alvins:** None. **J. Sutphen:** None. **M. Lang:** None. **S. Davis:** None. **T. Hanania:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: VA Mid-Atlantic Mental Illness Research, Educational and Clinical Research Center Grant (WCW, RMR)

Duke University Dean's Research Fellowship (NLN)

Howard Hughes Research Fellowship (MWP)

Department of Defense Grant W81XWH-08-2-0110

Title: Acetylcholinesterase (AChE) heterozygous mice present with a postraumatic stress disorder-like phenotype

Authors: *K. SMITH[1,2], R. M. RODRIGUIZ[3], J. S. COLVIN[2], M. W. PEASE[2], N.-L. NGUYEN[2], C. KIM[2], J. J. WILKINS[2], D. E. WILLIAMSON[2], W. C. WETSEL[3,2,4,5]; [1]Neurosci., UT Hlth. Sci. Ctr. San Antonio, Durham, NC; [2]Psychiatry and Behavioral Sci., [3]Mouse Behavioral and Neuroendocrine Analysis Core Facility, [4]Neurobio., [5]Cell Biol., Duke Univ., Durham, NC

Abstract: The neurotransmitter, acetylcholine (ACh), is inactivated primarily by the enzyme acetylcholinesterase (AChE). Blockade of AChE has been implicated in anxiety-like behaviors in both rodents and humans. Here, heterozygous (HET) AChE mice were behaviorally assessed in tests for anxiety, hyperarousal, and fear learning and extinction. These behaviors were assessed also following treatment with fluoxetine, a selective serotonin reuptake inhibitor, and diazepam, an anxiolytic. Compared to their wild-type (WT) littermates, HET mice spent less time in the open areas of the elevated zero maze and they showed augmented startle responses to white noise stimuli (80-120dB). Although diazepam exerted no effect on zero-maze behaviors, acute fluoxetine treatment dose-dependently alleviated both the zero-maze and the hyperarousal behaviors. Following auditory fear conditioning, HET mice exhibited enhanced freezing behaviors to both context and cued testing compared to their WT controls. During extinction, HET mice demonstrated a significant delay in reducing their freezing responses to the

conditioned stimulus when tested over 8 days. Separate groups of mice were subjected to fear conditioning and then were treated for 15 days with the vehicle or fluoxetine. Examinging for contextual and cued fear conditioning, freezing responses were reduced in the fluoxetine-treated HET mice relative to the HET vehicle controls and their responses were not differentiated from those of their WT littermates. These findings show that AChE-deficient mice present with heightened anxiety- and posttraumatic stress disorder- like behaviors and these responses can be alleviated with fluoxetine. Together, our results suggest that the AChE HET mice may represent a useful animal model to examine the biological mechanisms that may underlie these abnormal behaviors.

Disclosures: **K. Smith:** None. **R.M. Rodriguiz:** None. **J.S. Colvin:** None. **M.W. Pease:** None. **N. Nguyen:** None. **C. Kim:** None. **J.J. Wilkins:** None. **D.E. Williamson:** None. **W.C. Wetsel:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: DA017949

Title: Acute nicotine enhances spontaneous recovery of contextual fear and changes c-fos early gene expression in infralimbic cortex, hippocampus, and amygdala

Authors: *J. TUMOLO[1], B. GARRETT[2], M. G. KUTLU[2], E. HOLLIDAY[2], T. J. GOULD[2]; [1]Psychology, [2]Temple Univ., Philadelphia, PA

Abstract: Post-traumatic stress disorder (PTSD) is a devastating disorder with a lifetime prevalence of approximately 7% in adult Americans. Exposure therapy, which attempts to extinguish cues and contexts that trigger fear responses, is an effective treatment to reduce fear-related symptoms of PTSD. However, relapse of these symptoms following successful exposure therapy is fairly common. In rodents, spontaneous recovery (SR), where extinguished fear response resurfaces following extinction treatment, is used as a model of fear relapse. Previous studies from our lab identified nicotine as a strong mediator of fear extinction but the nicotinic modulation of fear relapse is unknown. Therefore, in the present study, we investigated the effects of acute nicotine administration on spontaneous recovery of contextual fear in C57BL6/J mice. In Experiment 1, mice were trained in contextual fear, given extinction, and one week later tested for SR immediately following administration of either acute nicotine (0.18 mg/kg) or

saline. Mice given nicotine showed significantly higher levels of SR than those given saline. In Experiment 2, in order to test whether acute nicotine's effects are specific to the retrieval of extinction memories, we trained mice using the same procedure but this time subjects did not receive extinction treatment. Again, mice were given either acute nicotine (0.18 mg/kg) or saline prior to memory recall test 12 days later. Our results demonstrated that mice administered acute nicotine showed comparable freezing levels to those administered saline, suggesting that acute nicotine selectively modulates retrieval of extinction memories. Finally, we employed a *c-fos* immunohistochemistry experiment in order to identify the brain regions responsible for the enhancing effects of acute nicotine on SR. The results of this experiment showed that *c-fos* immunoreactivity cells were significantly increased in the ventral hippocampus and basolateral amygdala and significantly decreased in the infralimbic cortex compared to saline-treated mice. Overall, our results show that acute nicotine may negatively affect retrieval of extinction memories and therefore augment fear relapse by altering the activation patterns of key brain regions in the fear extinction circuitry.

Disclosures: J. Tumolo: None. **B. Garrett:** None. **M.G. Kutlu:** None. **E. Holliday:** None. **T.J. Gould:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.20/KKK1

Topic: G.06. Post-traumatic Stress Disorder

Support: DoD award W81XWH-12-2-0048

Title: mGluR5 mediates both resilience to traumatic stress and relapse to cocaine seeking.

Authors: *J. SHALLCROSS, L. KNACKSTEDT, M. SCHWENDT;
Univ. of Florida, Gainesville, FL

Abstract: Post-traumatic stress disorder (PTSD) develops in a subset of people exposed to trauma. Complicating successful treatment of PTSD is comorbidity with substance use disorder. In this study we utilized a novel rodent model of comorbid PTSD-cocaine addiction that separates rats into trauma-resilient (RES) and susceptible (SUS) cohorts prior to cocaine exposure. First, rats underwent a single 10 minute exposure to the predator odor 2,4,5-trimethylthiazoline (TMT) and were then classified as RES or SUS using cut-off behavioral criteria based on behavior in both the elevated plus maze (EPM) and acoustic startle response (ASR) tasks. SUS, RES, and controls rats went through cocaine self-administration followed by

extinction training and a cue-primed reinstatement test. We found that drug-taking behavior did not differ among groups, however SUS animals showed delayed extinction learning and significantly higher reinstatement than both RES and control animals. In a subset of animals that experienced only TMT exposure, we performed RT-PCR analysis of amygdala and PFC mGlu5 receptor mRNA and found significantly higher expression in RES as compared to both SUS and control rats, suggesting a potential role of increased mGlu5 in encoding resilience to trauma. We then assessed the efficacy of compounds to enhance extinction of both operant responding and conditioned fear and to reduce cocaine-seeking in a reinstatement test. Rats underwent daily extinction of both the TMT context and the operant response and were treated with vehicle CDPPB, a positive allosteric modulator (PAM) of mGlu5, during the first five days of extinction. During the last 5 days of extinction, rats received either vehicle or ceftriaxone, an antibiotic reliably shown to reduce cocaine relapse, immediately after the extinction session. CDPPB enhanced operant extinction learning in SUS rats. Ceftriaxone alone (without CDPPB) significantly reduced lever pressing during a cue-primed reinstatement test in both Ctrl and RES, but only in SUS rats that had received CDPPB previously. Preliminary analysis of fear response during extinction shows significantly reduced freezing in SUS rats treated with CDPPB compared to vehicle. Characterization of specific mGlu5 mediated circuitry involved in resilience may aid in the development of precise pharmacological interventions for individuals with trauma and stressor related disorders. Supported by: DoD award W81XWH-12-2-0048, Institute for Translational Neuroscience subcontract 8738.

Disclosures: J. Shallcross: None. **L. Knackstedt:** None. **M. Schwendt:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.21/KKK2

Topic: G.06. Post-traumatic Stress Disorder

Support: W81XWH-08-1-0661

Title: Effects of single prolonged stress, a ptsd model, on adult hippocampal neurogenesis and extinction-retention

Authors: *E. RODRIGUEZ[1], I. LIBERZON[2];
[2]Psychiatry, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Functionally, posttraumatic stress disorder (PTSD) has been linked to impairments in fear extinction retention and contextual processing, dependent on intact prefrontal cortex-

hippocampus circuitry. Hippocampus is critical for processing of contextual information, and fear extinction depends on the context. Interestingly, PTSD patients have been reported to have hippocampus functional and volumetric abnormalities. At the cellular level, adult hippocampal neurogenesis plays a role in key hippocampal functions. Furthermore, neurogenesis is sensitive to stress suggesting a link between traumatic stress, hippocampal neurogenesis and deficits in contextual processing. Here we investigate if our PTSD model- single prolonged stress- affects adult hippocampal neurogenesis and how it relates to context-dependent fear processing. Experiment 1- all rats were injected with BrdU twice per day for four days, then left undisturbed for 21 days. Rats were then assigned to either Controls or SPS. SPS consists of two hours of restraint, immediately followed by 20min force swim, 15min rest, exposure to ether until loss of consciousness, then seven days undisturbed. After, all rats are fear conditioned (FC) to a tone in context A , the next day they are exposed to the tone until fear is extinguished (FE) in context B, following day they are tested for recall (ER) by exposing to the same tone in context B. Experiment 2- In addition to procedures from Experiment 1, rats were given access to environmental enrichment for seven days following SPS, followed by FC, FE and ER during the next three days. Experiment 3- Rats were given access to locked or unlocked running wheels for 24days, exposed to either SPS or undisturbed, injected with BrdU for four days, followed by FC, FE and ER during the next three days. Brains were fixed and processed for immunohistochemistry using DAB or fluorescence for BrdU alone or co-labeling with NeuN. SPS decreased the number of BrdU+ cells in the dentate gyrus of the hippocampus. Results of the effects of environmental enrichment or voluntary exercise are pending. These results suggest that SPS exposure may lead to a decrease in hippocampal neurogenesis and that the degree to which it is reduced may play a direct role in fear extinction learning.

Disclosures: E. Rodriguez: None. **I. Liberzon:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.22/KKK3

Topic: G.06. Post-traumatic Stress Disorder

Title: Exploring novelty-seeking behaviours and amphetamine sensitization in rats exposed to single prolonged stress

Authors: *K. THIRUMAL[1], P. KENT[3], C. CAYER[3], J. JAMES[3], H. ANISMAN[2], Z. MERALI[3];

[1]Neurosci., [2]Carleton Univ., Ottawa, ON, Canada; [3]Royal Ottawa Inst. of Mental Hlth. Res., Ottawa, ON, Canada

Abstract: Recent epidemiological studies have reported high comorbidity rates of post-traumatic stress disorder (PTSD) and substance use disorders (SUD), however the underlying neuronal mechanism(s) contributing to this relationship remains largely unknown. Chronic substance abuse has been linked to neural modifications in reward pathways, particularly the mesolimbic dopaminergic pathway, resulting in profound alterations in the incentive value of rewarding stimuli. Single prolonged stress (SPS) is a validated preclinical model of PTSD in rats. The objective of the current study was to assess the effects of SPS on behavioral alterations linked with the dopaminergic system. To investigate this, male Sprague-Dawley rats were exposed to SPS or no stress and then assessed at least one week later for locomotor activity, novel object exploration (NOE) and pre-pulse inhibition (PPI) with or without *d*-amphetamine (AMP) (0.5 mg/kg) administration. Results showed that AMP-induced hyperactivity was significantly increased in rats previously exposed to SPS. In addition, in the NOE paradigm, SPS rats spent significantly more time in the center of the arena, had a higher number of entries into the center, and had a higher center-periphery ratio. Rats treated with AMP spent more time exploring the novel object, an effect enhanced by previous SPS exposure. Together, these findings suggest enhanced exploratory and novelty seeking behavior in rats previously exposed to SPS which may be indicative of greater impulsivity. In addition, these findings also suggest a cross sensitization between AMP and SPS which may have implications for the relationship between PTSD and SUD.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.23/KKK4

Topic: G.06. Post-traumatic Stress Disorder

Support: ONR

Title: Post-stress glucose eliminates PTSD-like symptoms following traumatic stress in rats: Temporal constraints

Authors: *N. SMITH, M. A. CONOSCENTI, T. R. MINOR;
UCLA, Los Angeles, CA

Abstract: Exposure to uncontrollable traumatic stress in the learned helplessness procedure results in PTSD-like symptoms during later testing, including exaggerated fearfulness to a mild stressor and a transition to an unresponsive state termed *conservation-withdrawal*. Symptoms are eliminated when stressed rats are given access to a concentrated glucose solution following the trauma. Rats had access to glucose for 18 hours after traumatic stress in the original study of glucose prophylaxis (Minor & Saade, 1996). Three experiments are reported that assessed any temporal constraints on the benefits of post-stress glucose ingestion.
Experiment 1 assessed temporal differences in fluid consumption in stressed and nonstressed rats during the 18 hours following stress induction. Rats were exposed to 100, 1.0 mA inescapable tail shocks (S) or simple restraint (R) in tubes during a 1.83 hours stress-induction session. Rats in each of the stress condition had 18 hours free access to glucose (G: 40% w/v) or water (W) in their drinking bottles to complete a 2x2 factorial design. Contacts were recorded when a circuit was completed between the water spout and the grounded floor of a rat's metal cage. Rats were exposed to 5, five-sec fixed duration footshocks in a shuttlebox 24 hours after stress induction. Unconditioned shuttle crossing to the shocks and intertrial behavioral freezing (a measure of fear) were scored during the test.
Peak fluid consumption occurred at the onset of darkness in all groups. However, S and R groups ingested significantly more fluid in the glucose condition during the first three hours following stress than did their water-control counterparts. Total fluid intake for the 18-hour post-stress period did not differ significantly among groups. Post-stress glucose ingestion eliminated the exaggerated fearfulness that normally occurs following traumatic stress, and eliminated the transition to conservation-withdrawal as measured by unconditioned shuttle crossings.
Experiment 2 varied time (1, 3, 6, or 18 hours) that glucose or water was available immediately following shock or restraint. Test performance improved in all groups given at least 3 hours access to glucose. Experiment 3 assessed how long after stress access to glucose could be delayed to prevent performance deficits. Groups that received access to glucose within 3 hours of trauma improved test performance compared to 6 hours after trauma.
These experiments provide additional evidence that post-stress glucose is a simple and effective method of preventing behavioral pathology following traumatic stress and identify important constraints when the manipulation is likely to be beneficial.

Disclosures: N. Smith: None. **M.A. Conoscenti:** None. **T.R. Minor:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant MH094835

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Title: IGFBP2 induces resilience to stress in rats via a novel non-IGF1 or AMPA receptor dependent mechanism

Authors: *J. S. BURGDORF[1], E. M. COLECHIO[2], N. GHOREISHI-HAACK[2], A. L. GROSS[2], X.-L. ZHANG[3], P. L. STANTON[4], R. L. KROES[2], J. R. MOSKAL[1,2];
[1]Falk Ctr. for Mol. Therapeutics, McCormick Sch. of Engin., Northwestern Univ., Evanston, IL; [2]Aptinyx Inc., Evanston, IL; [3]New York Med. Col., Valhalla, NY; [4]New York Med. Col., Evanston, IL

Abstract: Growth factors play an important role in both resilience to stress and positive affect. Insulin-like growth factor 1 (IGF1) is the only growth factor that has shown antidepressant properties in human clinical trials and is associated with positive affect. In animal studies, Insulin-like growth factor binding protein 2 (IGFBP2) has been shown to have both IGF1-dependent and IGF1-independent effects and is upregulated by positive affect induced by rough-and-tumble play in rats. We evaluated the ability of IGFBP2 to reverse stress-induced behavioral deficits using a rat chronic unpredictable stress paradigm. We tested the dependence of IGFBP2 effects on IGF1- and AMPA receptor activation using specific receptor antagonists. In addition, we measured dendritic spine morphology in the hippocampal dentate gyrus and medial prefrontal cortex 24 hrs after *in vivo* dosing. IGFBP2 (0.3-10 µg/kg i.v.) was 100 times more potent than IGF1 at reducing immobility in the Porsolt forced swim test. Unlike IGF1, the behavioral effects of IGFBP2 were not blocked by the IGF1-receptor antagonist JB1, or by the AMPA-receptor antagonist NBQX. A single dose of IGFBP2 (1 µg/kg IV) reversed stress induced deficits in Porsolt, novelty induced hypophagia, sucrose preference, and ultrasonic vocalization assays. Both IGF1 and IGFBP2 increased the density of mature dendritic spines in both medial prefrontal cortex and hippocampus 24 hrs post-dosing. Taken together, these data suggest that IGFBP2 induces resilience to stress via a novel IGF1 receptor-independent mechanism, and that IGFBP2 mimetics may have therapeutic potential for the treatment of neuropsychiatric disorders associated with chronic stress.

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Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.25/KKK6

Topic: G.06. Post-traumatic Stress Disorder

Support: University of Delaware Research Foundation award to DK

1P20GM103653

Title: The role of Akt signaling in persistent fear expression in a rodent model of post traumatic stress disorder

Authors: *D. K. KNOX, T. DEPIETRO, J. STAIB, M. CHAMNESS, E. MOULTON; Psychology, Univ. of Delaware, Newark, DE

Abstract: Single prolonged stress (SPS) exposure results in extinction retention deficits in rodents and is often used to model post traumatic stress disorder in humans. Recent research we have conducted suggests SPS-enhancements in fear memory could contribute to extinction retention deficits in the SPS paradigm. Previous studies have found that SPS increases baseline levels of Akt in the hippocampus. Furthermore, Akt signaling modulates fear memory formation. These findings raise the possibility that SPS could enhance fear memory by enhancing Akt signaling in emotional circuits in the brain. In turn, this enhancement in fear memory could lead to extinction retention deficits in the SPS model. To test this hypothesis, we looked at the effects of SPS on the Akt signaling in emotional circuits by examining levels of phospho and pan PDK and Akt in SPS and control rats at baseline and after fear conditioning. After removal from the housing colony or after the end of Pavlovian fear conditioning, rats were euthanized and brains extracted and stored at -80 ºC. The medial prefrontal cortex (mPFC), amygdala (AMY), ventral

hippocampus (vHipp), and dorsal hippocampus (dHipp) were then dissected from whole brains and western blot used to determine protein levels in all brain regions. While the study is ongoing, preliminary findings suggest that SPS enhances phosphorylated PDK levels in the mPFC and dHipp after fear conditioning. The initial results are consistent with the hypothesis that Akt signaling has a role in mediating extinction retention deficits in the SPS paradigm, which raises the possibility that enhanced Akt signaling after stress exposure could contribute to persistent fear memory expression.

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Poster

456. Post-Traumatic Stress Disorder: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.26/KKK7

Topic: G.06. Post-traumatic Stress Disorder

Title: Effects of glucose on learned helplessness behavior with a novel control condition

Authors: *N. C. CHRISTIE[1], M. A. CONOSCENTI[2], T. R. MINOR[2];
[1]Psychology, UCLA, Woodland Hills, CA; [2]Psychology, UCLA, Los Angeles, CA

Abstract: PTSD is a disorder that affects a wide range of people worldwide. A major contributing factor to the onset of PTSD is the inability of the hippocampus to consolidate the traumatic memory properly. Our intervention aimed to reinstate the consolidatory properties of the hippocampus by bringing it back "online." This memory can then be specified to the context of the trauma, leading to a reduction in the overgeneralization of fear to other contexts. In this experiment, 32 adult male Sprague-Dawley rats were separated into four groups of eight. The control group received no shock and had access to only water. The other three groups received either 18 hours of access to water, fructose, or glucose immediately following an acute stressor in the form of an uncontrollable tail shock administered over a time period of two hours. Fructose was used as a placebo control to determine that it is the unique properties of glucose that reduce learned helplessness behaviors and not simply a sweet tasting solution post-trauma. The shock glucose group received the same acute stressor of an uncontrollable tail shock administered over a time period of two hours, with 18 hours of access time to glucose immediately after trauma. The results of the post-trauma behavioral task show that there is no significant difference between the shock-fructose and shock-water groups. Both demonstrated the same levels of learned helplessness behavior in the shuttle box task. Consistent with previous

research in the lab, the shock-glucose and restraint-water groups group demonstrated significantly better learning in the post-trauma escapable shock shuttle box test.

Disclosures: **N.C. Christie:** None. **M.A. Conoscenti:** None. **T.R. Minor:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Topic: G.06. Post-traumatic Stress Disorder

Support: Bradley Department of Psychology O'Grady Award

Psi Chi Summer Research Grant

Title: Establishing a modified model of PTSD in adolescent rats

Authors: ***A. L. GARRISON**, E. N. WALSH, J. M. SMITH, T. E. KOELTZOW;
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Abstract: New diagnostic criteria for Posttraumatic Stress Disorder (PTSD) in children and adolescents highlight the need to better understand the neurophysiological adaptations that trauma might elicit during development. The single prolonged stress (SPS) rat model of PTSD reliably produces an enhanced fear response to traumatic cues and disrupted cortisol regulation similar to that typically observed in humans with PTSD (Wang et al., 2008; Knox et al., 2012). In addition, SPS has been shown to influence the response of rats to cocaine (Eagle et al., 2015), a finding that is relevant to reports of co-morbidity of PTSD with substance abuse disorders (Jacobsen et al., 2001). The current study aimed to investigate the possible long-term effects of exposure to a modification of the SPS model (two hours of restraint stress followed by 20 minutes of forced swim) during adolescent development (Postnatal Day 32-35 at time of induction). Dependent variables included spontaneous locomotor activity as well as responses to a black/white chamber and an open field. Data indicate that the modified SPS procedure failed to elicit robust alterations in the behavior of SPS adult rats compared to controls. In contrast, exposure of SPS to adolescent rats tended to decrease spontaneous locomotor activity, measured as total distance travelled during a one hour test (t (21) = 1.39, p = 0.09; Cohen's d = 0.59), compared to controls. Similar effects were observed when rats were tested 4 weeks later (t (21) = 1.68, p = 0.05; Cohen's d = 0.66). The results of the behavioral anxiety data were more complex. For example, total crosses in the open field tended to be lower among SPS rats (t (20) = 1.53, p = 0.07; Cohen's d = 0.45) when test two weeks after SPS, but this trend was absent when tested 4

weeks later (t (21) = 0.26, n.s.). By contrast, no statistically significant differences were observed in the black/white chamber two weeks after SPS (BW crosses: (t (21) = 0.62, n.s.), though SPS-exposed rats exhibited a statistically significant decrease in exploration of the black/white chamber when tested 4 weeks later (t (21) = 1.91, $p < 0.05$; Cohen's d = 0.81). Finally, although SPS-exposed adolescent rats tended to exhibit hypoactivity in a variety of measures, there were no statistically significant differences in response to a cocaine challenge (F (1,21) = 0.15, n.s.). Taken together, data results indicate that adolescent rats may be more sensitive to stress compared to adult rats. On-going research aims to identify factors that may correspond to vulnerable vs. resilient individual responses to trauma.

Disclosures: A.L. Garrison: None. **E.N. Walsh:** None. **J.M. Smith:** None. **T.E. Koeltzow:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 456.28/KKK9

Topic: G.06. Post-traumatic Stress Disorder

Support: VA Merit Award 2I01BX001075-04

Title: Combined single prolonged stress (SPS) and CO_2 inhalation as a novel model of comorbid post-traumatic stress disorder (PTSD) and panic disorder associated behaviors in mice

Authors: *K. M. MCMURRAY[1,2,3], J. D. SCHURDAK[2,3], L. L. VOLLMER[2], R. SAH[2,3]; [1]Psychiatry, Univ. of Cincinnati- Reading Campus, Cincinnati, OH; [2]Psychiatry, Univ. of Cincinnati, CIncinnati, OH; [3]Cincinnati VA, Cincinnati, OH

Abstract: Post-Traumatic Stress Disorder (**PTSD**) and Panic Disorder (**PD**) are highly comorbid and debilitating psychiatric disorders. PTSD and PD have a lifetime prevalence of ~8% and ~5% in civilian populations, respectively, while the incidence is higher in combat veterans (PTSD ~13%; PD ~8%). Primary treatments for both disorders are limited by high abuse potential (benzodiazepines), delayed onset (SSRI/SNRI) and a lack of effect in a large proportion of patients. Additionally, comorbid PD and PTSD is associated with worse patient outcomes pointing to a need for understanding the underlying pathophysiology in order to develop novel pharmacotherapies and improve treatment outcomes. Despite a high prevalence and debilitating effects, the neurobiology and underlying mechanisms of comorbid panic-PTSD is largely unknown. Development of translational models simulating comorbid panic and PTSD-like behaviors is urgently needed. Low dose CO_2 inhalation selectively induces panic attacks in PD

subjects compared to healthy controls. Further, prior sensitivity to CO_2 is associated with worse PTSD symptoms, suggesting potential for common vulnerability factors. Previous studies in our lab have used rodent models of CO_2-inhalation evoked freezing (panic-like) and traumatic stress evoked startle and fear conditioning (PTSD-like) behaviors (Vollmer et al 2016; McGuire et al 2010; Schmeltzer et al, 2015). The current study investigated the emergence of panic- and PTSD-relevant behaviors in a conjunction CO_2-inhalation-single prolonged stress (SPS) model. We hypothesized enhanced fear and panic associated behaviors in mice exposed to both stress and CO_2 inhalation. Male BALB/C mice were exposed to SPS (restraint for 2 hr, 10 min forced swim and exposure to ether until losing consciousness) or were undisturbed. One week later, all mice were exposed to air or 5% CO_2 for 10 min and freezing behavior was scored. Following one week of recovery PTSD-like behaviors were assessed (acoustic startle and context fear conditioning). Prior SPS exposure altered CO_2 evoked fear-like behaviors and induced deficits in fear-extinction learning in a subgroup of SPS treated animals. Freezing to CO_2 was correlated with freezing during fear conditioning only within SPS treated mice, suggesting SPS exposure may interact with CO_2 inhalation to impact PTSD-relevant behaviors. Ongoing studies are further investigating potential sub-populations representing susceptibility or resistance in a larger cohort. Collectively, development of this novel model will advance our understanding of comorbid PTSD and PD.

Disclosures: K.M. McMurray: None. **J.D. Schurdak:** None. **L.L. Vollmer:** None. **R. Sah:** None.

Poster

456. Post-Traumatic Stress Disorder: Models

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Program#/Poster#: 456.29/KKK10

Topic: G.06. Post-traumatic Stress Disorder

Support: Roskamp Foundation

VA

Title: Chronic 'PTSD-like' effects (after 6months) in a mouse model of comorbid traumatic stress and repetitive mTBI

Authors: *M. ALGAMAL[1], J. O. OJO[1,2], M. OWENS[1], M. MULLAN[1,2], D. DIAMOND[3,4], F. CRAWFORD[1,2,3];
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Abstract: Background: Comorbid mTBI and PTSD can be clinically challenging to diagnose in humans, due primarily to the heterogeneity and clinical overlap shared by both conditions. This emphasizes the critical need to develop a relevant animal model whereby many variables can be effectively controlled. This work aimed to investigate the behavioral and neurobiological interrelationship between mTBI and traumatic stress in a mouse model.
Methods: Four groups of animals were examined in this study – sham, stress, repetitive-mTBI (r-mTBI) and stress+rmTBI. Briefly our Two separate cohorts were used in this study. Cohort (I) received chronic stress and/or r- chronic stress paradigm involved 21 days of unpredictable repetitive exposures to: danger-related predator odor (Fox urine, TMT) whilst under restraint, a daily social stressor involving unstable social housing with an alternate congener, and physical trauma in the form of five repeated inescapable footshocks on separate days. Animals receiving r-mTBI and stresssors were exposed to five closed head injuries 1hr after each inescapable footshocks. mTBI paradigm once at 3 months of age, while cohort (II) received this twice, at 3 and 5 months of age. A battery of behavioral tests for fear, anxiety, depression and spatial memory was conducted, in addition to molecular profiling of brain and plasma samples.
Results: Behavioral analyses at 2 weeks, 3months and 6 months post-exposure showed that stressed mice in both cohorts demonstrated significant weight loss, recall of traumatic memories, anxiety and depressive-like behavior when compared to sham mice. Interestingly repeated mTBI abrogated the stress related phenotype in the fear memory, and depression tests. Neuroendocrine studies implicate the noradrenergic system and the HPA axis as contributory factors for the observed differences between stress and sham mice.
Conclusion: Our results show that animals exposed to stressful trauma alone demonstrate some similar traits with the human condition as defined by DSM-V. Distinct and overlapping traits were observed between the mTBI and stress groups. We anticipate that our model will be a good platform to explore targeted biomarkers and treatment strategies in PTSD and repetitive mTBI.

Disclosures: **M. Algamal:** None. **J.O. Ojo:** None. **M. Owens:** None. **M. Mullan:** None. **D. Diamond:** None. **F. Crawford:** None.

Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH P20GM103442

NIH P50DA018165

R24AA020245

Title: Relationship between methamphetamine use, reinforcement and seeking in mice with high genotype-dependent methamphetamine intake.

Authors: *S. SHABANI[1], E. I. MOJICA[1], L. HELLMUTH[1], S. HOULTON[1], T. J. PHILLIPS[2,3];
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Abstract: Based on data for cocaine addiction, pattern of drug use in the form of binge/abstinence cycles is thought to influence drug seeking, i.e. craving, and vice versa. General cocaine use pattern in humans, however, is different from methamphetamine (MA) use pattern. We have developed a genetic mouse model for which genetic factors associated with pattern of MA use also influence other addiction relevant traits such as sensitivity to the rewarding, reinforcing and aversive effects of MA. The genetic model is comprised of mouse lines generated via selective breeding for higher (MAHDR) and lower (MALDR) amounts of MA intake in a two-bottle choice (water vs. MA dissolved in water) drinking procedure. In the current study, male and female MAHDR mice and their higher MA intake progenitor strain, DBA/2J (D2) mice, were used to explore the influence of binge/abstinence MA use on MA seeking and taking. Mice first engaged in an operant oral self-administration procedure in which MA was gradually faded in and saccharin gradually faded out and MA concentration was increased to 80 mg/l. Then a multiple bottle choice procedure was used in which half of the animals were given access to a 3:1 ratio of MA:water bottles and half were controls given access to 4 water bottles only. The MA access period was for an 18h period daily; water was available 24h per day. During the multiple bottle choice procedure, MA concentration was increased every 4d from 20 to 40 and then 80 mg/l, and then animals were given access every other day for another 5 trials. Between days of acute withdrawals from voluntary drinking, animals resumed operant oral self-administration for 5 trials that were paired with both discrete and contextual cues. Finally, an extinction phase in the operant procedure lasted 16 days followed by a cue-induced reinstatement test. In MAHDR mice, MA reinforcement behavior in females was significantly lower than in males; however, female MAHDR mice had higher MA intake than males during subsequent voluntary MA drinking. Sex had no significant effect in D2 mice. MA reinforcement behavior was significantly higher in MAHDR than D2 mice. Prior exposure in an operant procedure had minimal effect on subsequent voluntary drinking in MAHDR mice, but substantially attenuated voluntary MA intake in D2 mice. Compared to water controls, voluntary MA drinking did not influence MA reinforcement behavior during acute withdrawal or MA seeking in a cue-induced reinstatement test in either genotype. Our results indicate that genotype and sex play important roles in MA use patterns, reinforcement and seeking traits, and the impact of MA experience on subsequent MA intake.

Disclosures: S. Shabani: None. **E.I. Mojica:** None. **L. Hellmuth:** None. **S. Houlton:** None. **T.J. Phillips:** None.

Poster

457. Amphetamines: Behavioral Studies

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Program#/Poster#: 457.02/KKK12

Topic: G.08. Drugs of Abuse and Addiction

Support: T32 DA007262-23

R01 DA025922

DOD W81XWH-12-2-0048

Title: Context-independent effects of footshock on drug-seeking

Authors: *C. PIZZIMENTI[1,2], T. NAVIS[2], K. M. LATTAL[2];
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Abstract: Fear-related disorders and substance use disorders are highly comorbid. Even following long periods of abstinence, comorbid individuals have high rates of relapse to drugs of abuse, especially in response to cues previously paired with drug. Previous attempts to characterize the role of fear on reinstatement have administered both the drug and the stressor within the same environment. Therefore, little is known about how fearful experiences in a specific context cause persistent changes in drug-seeking behavior in other contexts. To address this we examined the effects of massive footshock in one context on cue-induced drug-seeking for methamphetamine in another context. In Experiment 1, animals were trained to self-administer methamphetamine in Context A. On day 8 of self-administration (16 total sessions) animals received either a battery of footshocks in Context B or exposure to that context only, and were then allowed to continue daily self-administration sessions in Context A. Animals that received shock reinstated significantly more than controls to drug-associated cues and took significantly longer to extinguish lever pressing following reinstatement. In Experiment 2 the battery of footshocks were administered in Context B before acquisition of self-administration in Context A. Animals that received footshock showed significantly elevated responding during cue-induced reinstatement. In a separate group of animals corticosterone (CORT) levels were measured once per week for two weeks prior to footshock, immediately following footshock, and once per week for five weeks after footshock. These time points were chosen because it was at approximately five weeks following footshock that enhancements in cued-reinstatement were observed in Experiments 1 and 2. Animals also underwent a dexamethasone (DEX) test to assess the response of glucocorticoid receptors. CORT was significantly elevated immediately following footshock relative to controls, but there were no persistent differences between groups, and there was no reliable difference between groups in response to DEX. Taken together, these

results suggest that this novel, translatable model replicates the human condition in which exposure to a massive stressor in a specific context produces long-term changes in drug-seeking behavior in non-fear associated contexts. These data also suggest these changes are not due to long-term alterations to the HPA axis itself, but are likely due to changes at the neurobiological or circuit level.

Disclosures: **C. Pizzimenti:** None. **T. Navis:** None. **K.M. Lattal:** None.

Poster

457. Amphetamines: Behavioral Studies

Location: Halls B-H

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Program#/Poster#: 457.03/KKK13

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA034389

Title: Examination of sex dependent neural substrates correlated with meth triggered reinstatement in rats

Authors: *S. T. PITTENGER[1], S. CHOU[2], S. T. BARRETT[1], O. D. LOH[1], R. A. BEVINS[1]; [1]Univ. of Nebraska-Lincoln, Lincoln, NE; [2]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Methamphetamine (meth) use results in a heavy economic and societal burden, costing the United States an estimated 23 billion dollars annually. Meth dependence is often characterized by persistent and chronic relapse to drug use. A relapse can occur weeks or even months after the last drug use, often resulting in periods of sustained meth use. Relapse after long periods of abstinence remains a major impediment to the treatment of meth use and drug addiction. Notably, nearly 45% of individuals that do seek treatment for meth addiction are women. Given the high rates of meth addiction in women, the role of sex in meth relapse remains understudied. The limited animal research that has investigated sex differences on meth-taking has illuminated notable differences between the sexes. Preliminary work in our lab investigating sex-differences in meth reinstatement has shown that females show greater reinstatement induced by a meth priming injection. This study identified sex-dependent neural substrates correlated with meth-triggered reinstatement using immunohistochemistry. Male and female Sprague-Dawley rats were surgically implanted with indwelling jugular catheters. Half of the rats were then trained to self-administer meth; the other half self-administered saline infusions during 21 daily sessions (2h). Rats were then given 12 extinction sessions. Twenty-four hours after the last extinction session, rats received reinstatement testing. Half of the rats received a meth-prime (0.3 mg/kg, SC) injection and half of the rats received a saline injection.

This design resulted in 4 separate groups for each sex (SalSA/SalT, SalSA/MethT, MethSA/SalT, MethSA/MethT) allowing for careful investigation of brain regions correlated with meth-triggered reinstatement. Brains were harvested following the reinstatement session and cFos immunoreactivity was measured in 20 brain regions. Meth triggered reinstatement in both sexes and this effect was more robust in females compared to males. Fos activation was significantly increased following meth-primed reinstatement in the cingulate cortex area 1, lateral orbitofrontal cortex, prelimbic cortex, caudate putamen, nucleus accumbens core and shell, central nucleus of the amygdala. Significant sex differences were also found with females showing greater cFos immunoreactivity. These sex differences were found to be greatest in rats that received a meth-prime following meth self-administration (MethSA/MethT) and in brain regions associated with drug addiction (e.g., cingulate cortex area 1, lateral orbitofrontal cortex, caudate putamen, nucleus accumbens core).

Disclosures: **S.T. Pittenger:** None. **S. Chou:** None. **S.T. Barrett:** None. **O.D. Loh:** None. **R.A. Bevins:** None.

Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: Intramural Research Program, NIDA, NIH, DHHS

Title: Cue-induced relapse to methamphetamine seeking in compulsive methamphetamine takers and abstinent rats

Authors: *I. N. KRASNOVA, N. TERRY, M. MCCOY, B. LADENHEIM, J. CADET;
Mol. Neuropsychiatry Res. Br., NIDA, NIH, DHHS, Baltimore, MD

Abstract: The treatment of methamphetamine (METH) addiction is associated with a high rate of relapse to drug use even after long periods of abstinence. The high degree of recidivism is probably related to METH-induced neuroadaptations in the brain. Here, we studied differences in cue-induced relapse in rats that continue to self-administer METH compulsively and those that became abstinent in the presence of contingent footshocks. We trained rats to self-administer METH (0.1 mg/kg/ infusion) for 9 h/day for 20 days. Subsequently, 50% of the METH infusions were punished by mild electric foot-shocks (0.18 ->0.36 mA, 0.5 sec) for 10 days. A control group of rats self-administered saline. During training, rats showed binge patterns of METH self-administration, with the first hour intake gradually escalating over time. Total METH intake also

escalated over time. During shock phase, approximately 55% of animals reduced METH self-administration to about 20% of pre-shock level (shock-sensitive rats), whereas the remaining animals continued to self-administer the drug (>50% of pre-shock level) in spite of electric foot-shocks (shock-resistant rats). Importantly, we did not find significant differences in drug intake during self-administration training between the shock-resistant and shock-sensitive rats. Next, we conducted extinction tests on withdrawal days 2 and 30 in the presence of METH-associated cues. We found increases in cue-induced reward seeking on withdrawal day 30 in comparison to withdrawal day 2 in both, shock-resistant and shock-sensitive rats. However, shock-resistant rats showed significantly higher METH seeking than shock-sensitive animals. Our results show that escalation of METH intake occurs in rats that become compulsive drug takers and in those that develop abstinence in the presence of adverse consequences. These observations are consistent with clinical literature. Our findings also revealed that compulsive METH takers showed a greater relapse to drug seeking than abstinent rats.

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Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant NS076517

NIH Grant MH096463

Title: Effects of Ro5-4864 on methamphetamine self-administration in male and female rats

Authors: ***G. F. GUERIN**[1], S. M. HAROLD[1], S. R. PORTER[1], C. D. SCHMOUTZ[1], G. LI[2], J. M. COOK[2], N. E. GOEDERS[1];
[1]LSUHSC-S, Shreveport, LA; [2]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Methamphetamine abuse continues to be a serious and growing problem with no approved treatment. We have previously demonstrated differential effects of benzodiazepines on various methamphetamine-related behaviors based upon their relative affinities for either the "central" benzodiazepine (BZD) binding site associated with the $GABA_A$ receptor or the "peripheral" BZD binding site associated with the translocator protein of 18kDa (TSPO). Specifically, oxazepam, which binds to both receptors, decreases methamphetamine self-

administration, conditioned place preference and drug discrimination. In contrast, alprazolam, which binds only to the central BZD binding site, augments the effects of methamphetamine at certain dose combinations. The current experiment was designed to investigate the effects of Ro5-4864 on methamphetamine self-administration in rats. Ro5-4864 is a potent selective ligand for TSPO that does not bind to the $GABA_A$ receptor complex and lacks typical "central" benzodiazepine-like effects. Male and female Wistar rats were trained to self-administer methamphetamine (0.05 mg/kg/infusion) during daily two-hour sessions. After responding stabilized, several doses of Ro5-4864 were administered intraperitoneally 30 minutes before the start of the sessions. Self-administration was dose dependently decreased in both males and females; however, female rats were more sensitive to these effects. The TSPO receptor has an integral role in the ability of certain benzodiazepines to decrease methamphetamine self-administration and could be important in developing novel treatments for stimulant addiction.

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Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: N01-DA78872

T32 AG020494

Title: Differential effects of ratio requirement on reinforcing efficacy of synthetic cathinone analogs of MDMA

Authors: *S. B. DOLAN, M. GATCH;
Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Novel psychoactive substances have been established as a prominent fixture in global drug culture, with synthetic cathinones being amongst the most prevalent of these substances. Although initially used as semi-legal alternatives to illicit recreational drugs, many synthetic cathinones have been diverted into "ecstasy" formulations *in lieu* of MDMA. The current study aimed to investigate the reinforcing effects of MDMA along with three synthetic cathinone analogs of MDMA with propyl-, butyl-, or pentylamine side-chains: methylone, butylone, and pentylone, respectively. Two separate groups of adult, male Sprague-Dawley rats were trained to

self-administer 0.05 mg/kg/infusion methamphetamine under a FR1 or FR10 schedule of reinforcement. Rats trained on the FR1 schedule underwent substitution testing under an FR1, whereas rats trained on the FR10 underwent substitution testing under a progressive ratio. Each test compound was robustly self-administered at similar rates under the FR1 schedule. Conversely, under the progressive ratio, there were substantial differences in reinforcing efficacy among the compounds. Methylone and butylone produced greater self-administration than vehicle controls, but less than methamphetamine controls; however, pentylone produced self-administration comparable to methamphetamine. MDMA, on the other hand, was not self-administered at a greater rate than the vehicle control. These results indicate that each of the test compounds serves as a reinforcer under a continuous schedule of reinforcement, but when the behavioral cost of reinforcement is increased under the progressive ratio, the reinforcing efficacy of these synthetic cathinones is positively associated with its side-chain length. Additionally, these data indicate that the cathinone analogs of MDMA may serve as more efficacious reinforcers than MDMA. Translationally, these data suggest that MDMA and the synthetic cathinone analogs are equally efficacious as substitutes for methamphetamine when access is unhindered, but when increased costs are imposed, synthetic cathinones with longer side-chains may be used as substitutes for psychostimulant-maintained behavior. Furthermore, the prevalence of these synthetic cathinone analogs of MDMA in “ecstasy” formulations may result in increased compulsive “ecstasy” use in turn leading to complications arising from acute intoxication as well as increased likelihood for chronic use not typically seen with MDMA.

Disclosures: S.B. Dolan: None. **M. Gatch:** None.

Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: DoD Grant W81XWH-12-2-0048

Title: Effects of oxytocin following traumatic stress on methamphetamine seeking in female rats.

Authors: *C. E. O'NEILL, R. J. NEWSOM, J. F. MCGINTY;
Inst. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous research has shown a strong link between posttraumatic stress disorder (PTSD) and substance use disorders (SUD). Stimulants, such as methamphetamine (METH), are

among the most highly abused by those with comorbid PTSD, and these patients typically exhibit a longer history of use and poorer overall treatment outcomes in comparison with individuals diagnosed with a SUD alone. Previous work from our lab has shown that male rats exposed to 5 days of TMT(2,4,5-dihydro-2,5-trimehylthiazoline) exhibit exacerbated levels of TMT-induced reinstatement (RST), but that 10 days of systemic oxytocin (OXT) administration prior to initiation of METH self-administration blocks RST only in TMT pre-exposed rats (Ferland et al., 2016). The goal of the present study was to determine whether these effects are similar in female rats. Thus, we exposed female rats to TMT or saline for 5 days followed by 10 days of OXT (1.0 mg/kg, i.p.) or saline. Plasma corticosterone (CORT) was significantly elevated and animals displayed increased anxiety in the open field following 15 min of TMT exposure on both the 1st and 5th day, with no evidence of habituation to the stressor. Plasma OXT levels were elevated following the 1st day of TMT exposure, but returned to baseline levels by the 5th day. Similar to males, TMT pre-exposed female rats injected with saline for 10 days prior to METH SA exhibited significantly increased stress-induced RST than saline pre-exposed rats injected with saline prior to METH SA. In contrast, female rats pre-exposed to saline followed by 10 days of OXT showed a significant decrease in stress-induced METH seeking compared to saline pre-exposed rats injected with saline prior to the initiation of SA. The same pattern was observed in cue-induced RST, indicating that OXT treatment following either TMT or saline pre-exposure suppressed METH seeking. Tissue collected following TMT-induced RST is currently being processed for alterations in mRNA expression for genes related to OXT and stress signaling in several brain regions.

Disclosures: C.E. O'Neill: None. **R.J. Newsom:** None. **J.F. McGinty:** None.

Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH; NIDA R15DA035435-01

NIH; NIDA R15DA035435-01S1

Title: Rearing condition alters the ability of ceftriaxone to attenuate cue and amphetamine reinstatement

Authors: *E. J. GARCIA, D. L. ARNDT, M. E. CAIN;
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Abstract: The glutamate homeostasis hypothesis of addiction proposes that excessive synaptic and extracellular glutamate levels promote drug reinstatement after extinction. After chronic psychostimulant self-administration, glutamate homeostasis is reduced by decreasing cysteine-glutamate exchanger (xCT) and primary glial glutamate transporter 1 (GLT1) expression. Ceftriaxone (CTX) restores glutamate homeostasis by upregulating xCT and GLT1, thereby reducing reinstatement. Rodents reared in an enriched condition (EC) show faster extinction learning and less drug-induced reinstatement when compared to rats reared in isolated (IC) or standard conditions (SC). The effect of rearing condition on glutamate homeostasis during reinstatement is not understood. To understand how rearing condition alters the function of GLT1 and determine how GLT1 is implicated in AMP reinstatement, rats were randomly assigned to vehicle (VEH) or CTX treatment after a 30 day rearing period in EC, IC, or SC conditions. Rats were then implanted with indwelling jugular catheters and allowed to self-administer AMP (0.1 mg/kg/infusion) on a FR1 schedule of reinforcement during 60-min sessions. AMP reinforcement was paired with a tone/light stimulus. After stable responding was achieved, rats were trained under extinction conditions such that active lever responding no longer presented AMP reinforcement or the tone/light stimulus. Beginning on the 6th extinction day, differentially reared rats were randomly assigned to receive daily injections of VEH or CTX (200 mg/kg ip) that continued through the last reinstatement test. After responding on the active lever was significantly reduced, rats were tested in a cue-induced reinstatement test where active lever responding resulted in the tone/light stimulus. Rats were trained in extinction and responding was reduced again prior to the AMP-induced (0.25 mg/kg s.c.) reinstatement test. During the AMP-induced reinstatement test active lever responding resulted in no programmed consequence. Results indicated that EC rats had a faster rate of extinction but CTX administration did not affect extinction learning. The cue-induced reinstatement test revealed that EC rats had less responding than IC or SC rats. Notably, CTX treatment reduced EC responding and increased IC responding in the first 10 minutes of the cue-induced reinstatement test. During the AMP-induced reinstatement test, CTX significantly reduced responding in SC rats but not EC or IC rats. These results demonstrate EC rearing protects against cue-induced reinstatement and that rearing condition changes the ability of CTX to restore glutamate homeostasis.

Disclosures: **E.J. Garcia:** None. **D.L. Arndt:** None. **M.E. Cain:** None.

Poster

457. Amphetamines: Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA041967

Title: Self-administration of psychstimulants via vapor inhalation in rats

Authors: *M. A. TAFFE[1], J. D. NGUYEN[2], S. A. VANDEWATER[2], M. COLE[3];
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Abstract: Addiction to methamphetamine (MA), cocaine and increasingly the cathinone derivative psychostimulants ("bath salts", "plant food", "flakka"), interferes with many aspects of personal health, vocational performance, interpersonal relationships and financial well-being. The consequences of stimulant addiction also strain legal and emergency medical resources throughout the US, making it a significant public health problem. Despite higher rates of treatment admissions for smoked versus other routes of cocaine administration and increasing proportions of MA users who smoke, there are no convenient models of inhalation self-administration available for rodent research. We have developed an e-cigarette based technology that can deliver locomotor stimulant doses of MA, 4-methylmethcathinone (mephedrone), 3,4-methylenedioxypyrovalerone (MDPV) or α-Pyrrolidinopentiophenone (alpha-PVP) to rats via inhalation. This study determined if inhaled psychomotor stimulants serve to reinforce operant behavior. Groups of rats were trained to nose poke for 2-min epochs of exposure to vaporized MA, MDPV, mephedrone, alpha-PVP or the propylene glycol (PG) vehicle. Stable or gradually increasing numbers of stimulant vapor epochs were obtained while responding for the PG vehicle alone gradually decreased across sessions. Responses on the drug-associated hole increased relative to the alternate hole, with 89% selectivity observed in some groups. Pretreatment with the dopamine D1-like receptor antagonist SCH23390 decreased responding for MA vapor. This study shows that rats will make nose poke responses to obtain access to psychostimulant vapor. This establishes a new model of intrapulmonary self-administration of drugs in rats which uses a technology (e-cigarette) that is increasingly being adapted in human users for non-nicotine drug use.

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Poster

457. Amphetamines: Behavioral Studies

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Sufficiency of dopamine receptor stimulation in the reinstatement of methamphetamine seeking

Authors: T. A. LARSON[1], M. C. WINKLER[1], *R. K. BACHTELL[2];
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Abstract: Methamphetamine (METH) continues to be a widely used psychostimulant, however the neurobiological mechanisms underlying it's seeking and relapse are still unclear. Previous data has shown that antagonism of dopamine receptor subtypes differentially affect METH reinforcement and relapse. Using a common rodent model of relapse, we assessed whether stimulation of dopamine receptor subtypes (D1, D2, and D3) are sufficient to induce METH seeking. Rats were trained to self-administer METH via lever press during daily 2-hr sessions on a fixed-ratio 1 schedule for 10 consecutive days. Extinction procedures commenced after 1 day of abstinence and lever pressing was extinguished throughout the 6 consecutive extinction sessions. The following pharmacologically developed agonists were administered via systemic intraperitoneal injection or directly into the nucleus accumbens core (NAc) prior to a reinstatement test session: SKF 81297 (D1 agonist), quinpirole (D2-like agonist), and 7-OH-DPAT (D3 agonist). Systemic administration of SKF 81297 produced a dose-dependent modest reinstatement of METH seeking. Systemic quinpirole produced a robust dose dependent reinstatement of METH seeking. Intra-NAc administration of SKF 81297 produced robust reinstatement, while quinpirole and 7-OH-DPAT were ineffective. These findings demonstrate distinct reinstatement patterns that is dependent on the route of administration and may aid in assessing the involvement of specific dopamine receptor subtypes in methamphetamine seeking.

Disclosures: T.A. Larson: None. **M.C. Winkler:** None. **R.K. Bachtell:** None.

Poster

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Title: The 5-HT_{1B} receptor agonists, CP 94,253 and zolmitriptan, attenuate the reinforcing and motivational effects of methamphetamine.

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Abstract: We previously found that the selective 5-HT_{1B} receptor (5-HT_{1B}R) agonist, CP 94,253, enhances cocaine self-administration (SA) during initial training but attenuates SA after 3 weeks of abstinence from cocaine. Here we examined if CP 94,253 and the mixed 5-$HT_{1B/1D}$R agonist, zolmitriptan, produce similar abstinence-dependent effects on methamphetamine (meth) SA. Male Sprague-Dawley rats were tested for the effects of CP 94,253 on meth reinforcement rates on both fixed and variable ratio (VR) 5 schedules and on a progressive ratio (PR) schedule of reinforcement. Similarly, we tested for the effects of zolmitriptan on meth reinforcement on a VR5 schedule. We found the typical inverted U-shaped meth SA dose-response function when rats were pretreated with vehicle on both low ratio schedules. CP 94,253 produced a downward shift of the descending limb of the dose-response function prior to abstinence. Post-abstinence, CP 94,253 produced a downward shift of the entire dose-response function. CP 94,253 also attenuated reinforcers obtained and breakpoints on the PR schedule compared to vehicle pretreatment both pre- and post-abstinence. Importantly, administration of SB 224,289, a selective 5-HT_{1B} receptor antagonist, blocked the attenuating effects of CP 94,253 on meth SA. The decreases in SA measures were not likely due to motor impairments, as CP 94,253 had no effect on locomotor activity. Interestingly, acute treatment of zolmitriptan attenuated meth SA pre- and post-abstinence similar to the effects of CP 94,253. In addition, the attenuating effects of zolmitriptan on meth SA following a period of abstinence were sustained after each of 3 more treatments given every 2-3 days during the resumption of meth SA. Thus, unlike the abstinence-dependent modulatory effect of CP 94,253 on cocaine SA, this study found that both CP 94,253 and zolmitriptan inhibit meth SA regardless of abstinence. These findings suggest that 5-HT_{1B}R agonists may have clinical efficacy as treatments for psychostimulant use disorders.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: MARC

RISE

Title: Rewarding effects of a methamphetamine and morphine speedball as assessed by ultrasonic vocalizations in rats

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Abstract: The combination of a stimulant and opiate is commonly known as a "speedball". The traditional speedball is a combination of cocaine and heroin, and became newsworthy for contributing to the deaths of notable celebrities, including John Belushi and Chris Farley. However, with other opioids and psychomotor stimulants gaining popularity, including methamphetamine (METH) and morphine (MOR), non-traditional speedball combinations have the potential to be increasingly abused. The current study assessed the behavioral effects of a METH/MOR speedball. Animals were tested in two behavioral paradigms: locomotor activity and ultrasonic vocalizations (USVs) since increases in locomotor behavior and 50 kHz USVs reflect increases in reward. Animals received saline (control), METH (0.3mg/kg), MOR (5.0 mg/kg), or a combination of METH and MOR (speedball). METH-treated animals had immediate and long-lasting increases in locomotor behavior, representing stimulating properties of the drug. MOR-treated animals showed a biphasic locomotor response, with an initial mild depression of movement followed by an increase. Activity in the speedball group was much greater than either drug alone, suggestive of a synergistic effect. When assessing USVs, METH produced a rapid increase in vocalizations, reflecting its powerful rewarding effects. Similar to past work, MOR did not induce 50 kHz vocalizations. In contrast to locomotor behavior, the speedball group did not show an increase in USVs; in fact, it appeared that MOR inhibited METH-induced USVs. The ability of MOR to inhibit METH-induced USVs suggests that opioids may play an active role in suppressing vocalizations. In this regard, evidence suggests that the nucleus ambiguus, a brainstem region populated with mu opioid receptors, is essential in

USV production. Thus, opioid activity in the nucleus ambiguus may inhibit the motor output pathway for the production of reward related USVs. The results replicate earlier work demonstrating synergistic interactions between METH and MOR in locomotor behavior, and suggest that 50 kHz USVs are not useful for assessing rewarding effects of opioids, alone or in combination with other drugs.

Disclosures: T.T. Towner: None. **A. Rocha:** None. **K.A. Trujillo:** None.

Poster

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Title: Complex behavioral interactions between dissociative drugs and methamphetamine

Authors: *A. ESCOBEDO, K. A. TRUJILLO;
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Abstract: Dissociative drugs such as phencyclidine (PCP, Angel's Dust) and ketamine (KET, Special K) are abused in the dance club and rave scene for their complex behavioral effects including hallucinations, delusions, excitement and reward. Similarly, methamphetamine (METH) is a powerful and highly addicting psychomotor stimulant that is used at dance clubs and raves. Because these drugs are use in the same settings, there is the potential for these drugs to be used simultaneously. Indeed, studies show that psychomotor stimulants, such as METH, are the drugs most likely to be used with dissociatives in dance club settings. Drug users often combine drugs of abuse to potentiate rewarding effects. This effect is most notable with a cocaine/heroin combination, known as a "speedball." Another reason for combining drugs is that one drug will reduce negative side-effects of the other. The present studies examined the behavioral effects of combining a dissociative drug with METH in Sprague-Dawley rats. The locomotor effects of KET (10.0 mg/kg s.c), PCP (1.0 mg/kg s.c), or MK-801 (0.1 mg/kg s.c), were assessed alone or in combination with METH (1.0 mg/kg s.c). It was hypothesized that a combination of METH with a dissociative would produce a greater stimulant effect than either drug alone. The doses of the dissociatives were selected because they are the lowest that produce a stimulant response. The dissociative was administered 15 minutes before METH and the

locomotor response was assessed in a Kinder Scientific Motor Monitor. Interestingly, although KET reduced METH-induced hyperlocomotion, the opposite was found for PCP and MK-801 - these drugs enhanced the locomotor response to METH. The results illustrate the complex effects of combinations of dissociatives with METH, and suggest that KET is unique in its ability to suppress METH-induced locomotor behavior. Moreover, the results raise questions as to why individuals abuse combinations of KET and METH. Whereas PCP or MK-801 enhances the response to METH, perhaps leading to increased reward, KET appears to reduce the rewarding effects of METH. Future research should explore the neural mechanisms that mediate these differing interactions and that explain the unique effects of KET in combination with METH.

Disclosures: **A. Escobedo:** None. **K.A. Trujillo:** None.

Poster

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Title: Altered effort expenditure and intact reward sensitivity for non-drug rewards in protracted drug withdrawal

Authors: *A. B. THOMPSON[1], J. GERSON[1], A. STOLYAROVA[1], A. BUGARIN[1], Z. GUTTMAN[1], J. JENTSCH[2], A. IZQUIERDO[1];
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Abstract: Humans and experimental animals exposed to drugs of abuse exhibit altered reward-seeking behavior that persists long after drug taking has ceased. We previously reported increased voluntary running behavior and increased effort for larger magnitude food rewards in rats withdrawn from methamphetamine, mAMPH (Thompson et al. 2015, Stolyarova et al. 2015). Here we set out to further investigate reward-seeking behavior in prolonged withdrawal. First we assessed reward sensitivity in Long-Evans rats that had undergone withdrawal from mAMPH or that were previously treated with saline (SAL). Following an escalating regimen of

exposure followed by 7-10 days of withdrawal, rats were given a choice between plain water and sucrose solutions of increasing concentrations (1, 2, 4, 8, 16, 32%). mAMPH- and SAL-pretreated animals preferred and consumed sucrose to the same extent. Similarly, when given free choice between lab chow and sugar pellets, all animals preferred the sugar pellets equivalently. We conclude from this that reward sensitivity and choice behavior are intact in protracted withdrawal when options have equal effort requirements. We then assessed willingness to exert effort in a progressive ratio task involving freely available chow as a competing reinforcer (Randall et al. 2012). Animals were pretrained on progressive ratio for 2-5 days prior to introduction of the chow. When trained to stable performance, mAMPH-withdrawn rats pressed the lever significantly less than SAL animals, though they consumed the same quantity of food. In order to assess the contribution of dopamine D2-containing medium spiny neurons (MSNs) to choice behavior, we administered the selective A2A agonist CGS 21680. Excitatory A2A receptors are selectively expressed on D2 MSNs, therefore this agonist mimics the effect of a phasic decrease in dopamine concentration. Whereas systemic administration of CGS 21680 resulted in a significant decrease in both lever pressing and chow consumption, microinfusions of CGS 21680 in the nucleus accumbens selectively reduced lever pressing while leaving chow consumption unaffected, suggesting that alterations in this striatal population contribute to the observed behavior. This reduced lever pressing was exhibited by both mAMPH and SAL-pretreated rats. mAMPH has previously been reported to decrease D2 function in the striatum (Jentsch et al., 2014), which may render the indirect pathway resistant to dopaminergic inhibition. Ongoing experiments are aimed at uncovering and reversing the mechanism by which mAMPH-withdrawn rats show reduced thresholds for action directed at the freely-available chow over the preferred, more costly option.

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Poster

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The Research Foundation of NY (1115107)

Title: Recovery effects on behavior and development during abstinence after chronic methylphenidate treatment

Authors: *D. FRICKE[1], A. VIJAYASHANTHAR[1], C. F. LOWINGER[1], L. B. JERMYN[1], L. S. ROBISON[2], M. HADJIARGYROU[4], D. E. KOMATSU[3], P. K. THANOS[1];
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Abstract: Methylphenidate (MP) has been prescribed for the treatment of Attention Deficit Hyperactivity Disorder. According to the International Narcotics Control Board, MP was dispensed at a record 2.4 billion doses in 2013. Most psychotropic drugs, show sudden and even extreme reversible effects after cessation of treatment. A previously established dual-dose drinking paradigm was used to mimic clinical drug delivery. Using an 8-hour-limited-access-drinking-paradigm, male Sprague-Dawley rats received MP solutions at 4 mg/kg MP (LD) or 30 mg/kg MP (HD) during the first hour, and 10 mg/kg MP (LD) or 60 mg/kg MP (HD) for the remaining 7 hours. This dosing paradigm produced peak serum concentrations at 8 ng/ml for the LD group and 30 ng/ml for the HD group. Chronic behavioral and physiological effects of these dual-dosages were assessed throughout three months of treatment and one month of abstinence, beginning in adolescence. Results have shown significant effects on food intake, body weight, locomotor activity, circadian rhythm activity, and anxiety/exploratory behavior during the 13 weeks of treatment. MP dose-dependently decreased body weight and food intake. HD MP resulted in hyperactivity limited during the dark cycle, decreased exploratory behavior, and increased anxiolytic behavior. During this abstinence, the average weight of HD MP remained the lowest but the rate at which it increased rose to that of the control group. Consumption for both treatment groups had returned to baseline. Therefore, MP's effect on body weight, consumption behaviors and appetite were reversible. The significant anxiolytic, exploratory and locomotor effects seen in HD MP during treatment, decreased and returned mostly to baseline with the cessation of MP treatment; once again suggesting that the effects of chronic MP administration are reversible. A decrease in total circadian locomotor activity, limited to the active (dark) cycle in HD MP was seen. This decrease brought the activity of HD MP down to control, even showing hypolocomotor behavior at certain time points, while the LD MP group remained hyper active during similar time points and similar percentages as when they were on treatment. Analysis for novel object recognition, social interaction and forced swim test are still in progress. Comparison to the female test group under the same conditions is also in progress.

Disclosures: D. Fricke: None. **A. Vijayashanthar:** None. **C.F. Lowinger:** None. **L.B. Jermyn:** None. **L.S. Robison:** None. **M. Hadjiargyrou:** None. **D.E. Komatsu:** None. **P.K. Thanos:** None.

Poster

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Title: Separating the agony from ecstasy: prosocial effects and neurotoxicity of R(-)-3,4-methylenedioxymethamphetamine in mice

Authors: *D. W. CURRY[1], L. L. HOWELL[2];
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Abstract: (+/–)-3,4-methylenedioxymethamphetamine ((+/–)MDMA) is an amphetamine derivative that became popular as a recreational drug (ecstasy) and therapeutic tool during the 1970's and early 1980's. Escalating use led to its prohibition but scientific interest in the drug has persisted due to its unique prosocial effects. Under clinical observation, volunteers report that (+/–)MDMA increases feelings of closeness towards others, empathy, and gregariousness. In addition to these acute effects, there is evidence of enduring therapeutic effects such as improved interpersonal functioning and significant symptom reduction in PTSD patients. An ongoing clinical trial is now investigating its potential as a treatment for autism. However, serious limitations remain to wider clinical use of (+/–)MDMA, including its abuse liability and suspected neurotoxicity. There is thus significant impetus to isolate the prosocial mechanisms of (+/–)MDMA from the neurotoxic and abuse related effects. We investigated the hypothesis that the left handed enantiomer of (+/–)MDMA, (–)MDMA, may retain the prosocial effects of racemic MDMA but lack neurotoxicity. We found that both (+/–)MDMA (7.8 mg/kg) and (–)MDMA (17 mg/kg) significantly increased murine social interaction, but only (+/–)MDMA produced stimulant-like side effects. Furthermore, unlike racemic MDMA, (–)MDMA did not induce hyperthermia or neuronal markers of toxicity such as reactive gliosis or decreased brain monoamine content. These results indicate that the prosocial effects of (+/–)MDMA are separable from the neurotoxic and locomotor stimulant effects. (–)MDMA has prosocial effects similar to racemic MDMA but does not increase locomotor behavior or induce markers of neurotoxicity in mice. Further evaluation of (–)MDMA is needed in other species, but these results suggest that it may be a more viable therapeutic than racemic MDMA.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Title: Effect of sigma 1 receptor antagonist PD 144418 on methamphetamine self administration in rats

Authors: *M. TAPIA, J. LEE, G. GEREAU, D. MILLER, M. WILL;
Univ. of Missouri., Columbia, MO

Abstract: Use of illicit drugs, such as methamphetamine is a growing health concern across the United States. A recent national survey of drug use and health by the National Institute on Drug Abuse found that approximately 5% of Americans 26 or older use methamphetamine. Methamphetamine, a psychostimulant, works by elevating extracellular monoamine neurotransmitters, including dopamine, via multiple pathways. One way is by increasing intracellular calcium levels via IP_3. In addition to its effects on dopamine, methamphetamine can also elicit effects via sigma receptors. Multiple studies have shown that its effects maybe partially mediated by sigma 1 receptors (σ1). σ1 receptor antagonists however can reduce these effects. For example, our lab has shown that locomotor induced activity of methamphetamine can be reduced with the administration of a σ1 antagonist PD-14418. The purpose of this study is to further investigate the role of σ1 receptor antagonist PD-144418, specifically on methamphetamine self-administration in rats. Rats were first food trained to familiarize them with the task via a fixed ratio schedule. Once the criterion for the fixed ratio was met, rats were implanted with intravenous catheters. Self-administration training of methamphetamine occurred on a fixed ratio (0.05 mg/infusion; 2-hour sessions 6 days per week). Active lever presses were recorded but not reinforced. When the criteria for FR2 was met, animals were given a single injection of PD-144418 and placed back into the operant chamber and allowed to self-administer methamphetamine. We predict that administration of PD-144418 will decrease the acquisition and maintenance of methamphetamine self-administration in rats by reducing the reinforcing nature of methamphetamine.

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Support: The University of St. Thomas Startup Laboratory Funding

Title: The effects of enriched environment on the response to methamphetamine in adolescent and adult mice

Authors: A. M. DAHLY, E. P. BAKER, E. C. MAGNUSON, *J. H. WEISS, *J. A. SIEGEL; Psychology and Neurosci., The Univ. of St. Thomas, Saint Paul, MN

Abstract: Methamphetamine (MA) has neurotoxic effects on the adult human brain that can lead to deficits in behavior and cognition. Long-term exposure to MA also alters the stress response and hypothalamus-pituitary-adrenal axis function. The rising rates of adolescent MA use make it imperative that we understand the long-term effects of MA exposure on the adolescent brain and how these effects may differ from those seen in adults. Rearing in an enriched environment reduces MA seeking behavior, MA self-administration, and MA cue-induced reinstatement in adult rodents. However, relatively little research has examined the effects of MA on adolescent behavior and how housing environment might alter the behavioral response to MA in adolescence. In order to assess age differences in the response to MA and the possible effect of housing environment on the response to MA, this research examines the long-term effects of MA exposure during adolescence or adulthood on behavior and plasma corticosterone levels in male C57BL/6J mice housed singly in standard housing conditions or housed in groups in enriched environments. Current experiments are ongoing to study the effects of adolescent or adult MA exposure and housing environment on behavior in the open field test, the Porsolt forced swim test, and the social interaction test to evaluate locomotor activity and risk-taking behavior, depression-like behavior, and social interaction behaviors, respectively. Plasma corticosterone levels will be measured following behavioral testing. Based on previous data, we expect adolescent and adult MA exposure to increase risk-taking behavior, increase depression-like behavior, decrease social behaviors, and decrease plasma corticosterone levels. Because the adolescent brain is still developing, we expect that the MA-induced behavioral and corticosterone alterations in adulthood will be less severe than those observed in adolescence. Furthermore, we predict that housing in an enriched environment will mitigate the effects of MA in both age groups, but that this effect will be more pronounced in the adolescent mice. These findings will contribute to a greater understanding of how MA alters behavior in an age group that has been relatively understudied and how an enriched environment might alter the response to MA.

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Poster

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Title: Systemic administration of the GABAA receptor antagonist bicuculline prevents the effects of the administration of the 5-HT1A receptor agonist 8-OH-DPAT on the discriminative signal of amphetamine in a conditioned taste aversion procedure

Authors: *F. MIRANDA-HERRERA, A. SANDOVAL-SÁNCHEZ, L. N. CEDILLO, J. C. JIMENEZ, A. I. BARRIENTOS-NORIEGA, R. I. RUIZ-GARCIA;
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Abstract: The mesolimbic dopamine (DA) system, specifically the projection from the ventral tegmental area (VTA) to the nucleus accumbens (nAcc), is an important locus for the production of the locomotor, reinforcing, rewarding, and discriminative stimulus effects of cocaine and amphetamine (AMPH). Evidence suggests that serotonin (5-HT) may regulate some of the behavioral effects of psychostimulants. Several studies indicate that 5-HT1A receptor subtype might play an important role in modulating some behavioral effects of AMPH. It has been reported that low doses of 8-OH-DPAT (DPAT), which preferentially stimulates 5-HT1A somatodendritic autoreceptors, reduces cocaine-induced locomotor activity. We demonstrated that the systemic administration of a low dose of DPAT (0.03 mg/kg) reduces the discriminative signal of AMPH. This effect may be due to the fact that DPAT administration stimulates somatodendritic 5-HT1A autoreceptors in the raphe nucleus to decrease the activities of serotonin neurons that project to the GABAergic interneurons in the VTA. Consequently, the decrease in the stimulation of 5-HT1B receptors, located on the presynaptic terminals of GABAergic interneurons that synapse with DAergic neurons, increases the GABA levels in the VTA and decreases the DA levels in the nAcc. If this suggestion is true, then the effect reported previously should be preventable if the GABAA receptors are blocked via the administration of a GABAA receptor antagonist. This study was designed to examine the effects of the systemic administration of the GABAA receptor antagonist bicuculline on the AMPH-induced discriminative signal using a conditioned taste aversion procedure. Additionally, the GABA levels in the VTA and the DA levels in the nAcc were also evaluated using an *in vivo*

microdialysis. After a group of rats learned AMPH-saline discrimination, AMPH was substituted with different doses of DPAT (0.003, 0.01 and 0.03 mg/kg), bicuculline (0.5, 1.0 and 3.0 mg/kg) or combinations of DPAT (0.03 mg/kg) + AMPH (1.0 mg/kg) or bicuculline (3.0 mg/kg) + DPAT (0.03 mg/kg) + AMPH (1.0 mg/kg). In separate groups of rats, the GABA and DA levels in the VTA and nAcc, respectively, were evaluated using *in vivo* microdialysis technique. We observed that DPAT did not substitute for AMPH, but when DPAT was administered in combination with AMPH, a dose-dependent decrease was observed in the AMPH cue. This effect was reversed by the administration of bicuculline. We also observed that DPAT increased the GABA levels in the VTA and decreased the DA levels in the nAcc. These data suggest that 5-HT1A somatodendritic autoreceptors might play a regulatory role in the discriminative AMPH cue.

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Poster

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CNPq

Title: Apomorphine induced post-trial increases or decreases in dopaminergic activation can reverse or enhance catalepsy conditioning induced by haloperidol

Authors: *M. P. CARRERA[1], R. J. CAREY[2], F. R. C. DIAS[1], L. R. OLIVEIRA[1], B. G. SANTOS[1], J. L. L. SILVA[1];
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Abstract: Haloperidol reduces behavioral activity and can induce catalepsy that can be conditioned as well as sensitized to contextual cues. Post-trial drug treatments can induce potent conditioned responses indicative of the efficacy of trace conditioning of drug effects. The present study determined the impact of post-trial low and high dose apomorphine treatments on conditioned catalepsy. A paired/unpaired Pavlovian conditioning protocol was used, where groups of rats were given 10 daily administrations of vehicle or haloperidol (1.0 mg/kg) either

paired or unpaired to catalepsy testing. Ten min. after the treatments, the rats were placed on a horizontal bar and catalepsy duration was recorded during a maximum period of 10 min. This was termed the haloperidol sensitization phase. After the end of this phase, a saline test was conducted to assess for conditioned catalepsy (conditioned catalepsy test 1). As expected, repeated haloperidol treatments induced sensitization and conditioning of a catalepsy response selectively in the paired group. On the next day, there was a haloperidol re-induction treatment. On the following day, conditioned catalepsy test 2 was carried out followed by immediate post-trial treatments, in which the groups were subdivided into 3 vehicle groups, 3 unpaired haloperidol groups and 3 paired haloperidol groups and were given one of three post-trial treatments (vehicle, 0.05 mg/kg apomorphine or 2.0 mg/kg apomorphine). On the next day, the conditioned catalepsy test 3 was performed. On the following day, a haloperidol challenge test was carried out. The high dose apomorphine post-trial treatment eliminated conditioned catalepsy and counteracted not only the sensitized catalepsy response but also the initial acute catalepsy response to haloperidol, whereas the low dose apomorphine post-trial treatment enhanced conditioned catalepsy and the haloperidol induced a sensitized catalepsy response. The same post-trial apomorphine treatments given to vehicle and unpaired groups were equivalent to post-trial vehicle treatments. These results demonstrated that the immediate post-trial interval even for a conditioned drug inhibitory association is malleable and that the conditioning can be modified by increases/decreases in activity of the dopamine system.

Disclosures: **M.P. Carrera:** A. Employment/Salary (full or part-time): Universidade Estadual do North Fluminense. 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; CNPq and FAPERJ. **R.J. Carey:** None. **F.R.C. Dias:** 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; FAPERJ. **L.R. Oliveira:** 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; UENF/PIBIC. **B.G. Santos:** 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; CAPES. **J.L.L. Silva:** 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; UENF/PIBIC.

Poster

458. Attention and Frontal Cortex

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR

NSERC

EJLB Foundation

Title: Attentional effects on network dynamics in local field potentials of primate lateral prefrontal cortex

Authors: *M. ABBASS[1], L. DUONG[1], A. SACHS[2], J. MARTINEZ-TRUJILLO[1];
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Abstract: Recent studies have investigated the brain's structural and functional connectivity networks, largely by using graph theory. Complex networks have been unveiled at the microscopic cellular level as well as at the whole-brain level through the use of neuroimaging. In particular, small world networks have been found across these scales. These are networks in which there is a high level of local clustering among nodes, with short paths that globally connect all the nodes in the network. Here, we investigate the presence small world networks among channels in a microelectrode array (MEA), representing small clusters of neurons. Two male macaque monkeys (*macaca Fascicularis*) were trained to attend to one of four stimuli presented in the different visual field quadrants. Local field potentials (LFPs) were recorded from a 96-channel MEA in the left lateral prefrontal cortex (Area 8A). Spectral power was obtained by complex wavelet convolutions for 34 frequencies, ranging from 4Hz to 250Hz. Mean power data in 200ms intervals from -400ms to 800ms around the cue were calculated for each frequency. Pearson's correlation was calculated between 32 channels of the MEA. Significant correlations ($p<0.05$) were used to obtain a clustering coefficient and path length. These parameters were used to quantify the small world property of the network. Significance was assessed with permutation testing. Higher clustering coefficients were observed in lower frequencies compared to higher frequencies during attention tasks. However, small world networks were observed in the higher frequency bands, during or shortly following the presentation of the cue. The most prominent small world network found across all trials was observed in the frequency band centered at 147Hz, 200-400ms following the cue (3.192, $p<0.001$). These results suggest that LFP high frequency oscillators within the LPFC have a small network architecture. This may reflect the intrinsic connectivity between neurons between cortical columns within the area.

Disclosures: **M. Abbass:** None. **L. Duong:** None. **A. Sachs:** None. **J. Martinez-Trujillo:** None.

Poster

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Title: Partially-segregated neuronal populations in the lateral prefrontal cortex encode attended and memorized visual features

Authors: *D. MENDOZA-HALLIDAY[1], J. MARTINEZ-TRUJILLO[2];
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Abstract: Visual attention and working memory are interrelated but separable cognitive functions known to depend on the lateral prefrontal cortex (LPFC). Whether they are subserved by the same or different neurons within this area remains controversial. To investigate this, we recorded LPFC neuronal activity in two macaques during two conditions: while they attended to the motion direction of a stimulus that remained visible during the entire trial, and while they maintained in working memory the direction of a stimulus after it disappeared. We found that some neurons mainly encoded the attended direction, others mainly encoded the memorized direction, and others encoded both to different extents. This form of mixed selectivity enabled a machine-learning algorithm to decode from the population activity, on a single-trial basis, the stimulus' direction and whether it was attended or memorized. Our results indicate that in LPFC, the neuronal populations encoding attended and memorized visual features do not fully overlap, but are instead partially segregated, providing a mechanism for the brain to distinguish between sensory and mnemonic representations.

Disclosures: **D. Mendoza-Halliday:** None. **J. Martinez-Trujillo:** None.

Poster

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Title: Chemogenetic inhibition of prefrontal projection neurons and attentional capacities in forebrain trkA-suppressed rats

Authors: *V. V. PARIKH, M. G. KUTLU, S. JOSHI, B. YEGLA;
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Abstract: The basal forebrain (BF) cholinergic system mediates essential aspects of attentional information processing and cholinergic atrophy is reported in Alzheimer's disease (AD). The prefrontal cortex (PFC) is a key area involved in top-down control of attention and cholinergic signaling. Additionally, disrupted prefrontal activity may be more detrimental in cholinergic pathology. Here, we utilized a chemogenetic approach involving DREADD (Designer Receptors Exclusively Activated by Designer Drugs) to study the consequences of prefrontal inhibition on attentional capacities in young rats with a normal or compromised cholinergic system. Because nerve growth factor signaling via tropomyosin kinase A (trkA) receptors is critical for cholinergic function, and this mechanism is disrupted in AD, cholinergic hypofunction was produced by BF trkA suppression. Wistar rats were trained in an operant sustained attention task (SAT) that consists of a random sequence of signal and non-signal trials divided into 3 blocks. Animals trained to criterion (≥70% correct responses), received bilateral infusions of AAV vector expressing trkA shRNA into the BF. Another cohort of animals received BF injections of a control AAV vector. Both groups of rats simultaneously received bilateral infusions of HA-tagged hM4D(Gi) under CaMKIIa promoter into the medial PFC. Following post-surgery recovery, the animals were kept on task for 4-weeks and then tested for performance in a distractor session (dSAT; flashing house light in the 2nd block). 45-min prior to the onset of testing session, the animals received an injection of clozapine-N-oxide (CNO; 1mg/kg i.p.) to inhibit the activity of prefrontal projection neurons. For control conditions, animals received the injection of a vehicle. TrkA knockdown *per se* did not affect the performance in the pre-distractor block but produced moderate impairments in the post-distractor block indicating disruption in cholinergic modulation of PFC activity. CNO injection did not affect the dSAT scores in rats with intact trkA receptors. However, contrary to our predictions, CNO-mediated Gi activation of CaMKII-positive neurons in the PFC reversed the detrimental effects of trkA suppression on performance under attentionally-taxing conditions. Together these data illustrate that prefrontal inhibition of subcortical and other cortical regions via projection neurons may

exert beneficial effects on attentional processes under conditions of cholinergic hypoactivity. Moreover, a balanced activity rather than hyperactivity of prefrontal projection neurons may be desirable to maintain cognitive performance in AD.

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Poster

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Title: Real-time tagging of visual, saccadic, spatial memory and attention prefrontal representations

Authors: ***S. BEN HAMED**[1], C. WARDAK[2], E. ASTRAND[3];
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Abstract: Seminal studies on the neurophysiology of the frontal eye fields (FEF) describe the existence, in this prefrontal cortical region, of visuo-motor neurons that respond both to a visual stimulus presented within their visual receptive field and to an eye movement executed towards that same spatial position. A high proportion of these visuo-motor cells also respond during the memory delay of a memory-guided saccade towards these same preferred spatial locations. As a result, these cells are proposed to subserve the prefrontal populational representations of spatial visual, oculomotor, memory and attentional information thanks to a *common* spatial code. In the present study, we use Brain-Machine Interface and machine-learning methods to quantify cortical spatial population information on single behavioral trials, to tag, with a high temporal resolution, these different neuronal processes and infer their corresponding population neuronal codes.The real-time decoding is performed onto simultaneous invasive multi-unit activity (MUA) recordings in the FEF, bilaterally (using two 24-contact linear multi-electrodes, one in each FEF), in two monkeys, while they are engaged in three different behavioral tasks. Task 1 is

a memory-guided saccade task designed to dissociate in time between visual-, memory/attention- and oculomotor-related processing. Task 2 is a manual response peripherally cued-target detection task, designed to dissociate in time between visual and spatial attention processing, in the absence of any eye movement. Task 3 is the same as task 2, except that the cue is a central cue. We show that prefrontal spatial information can be accessed through a simple linear decoding approach, suggesting a neural basis function encoding schema for this information. However, we also show that the neuronal population codes for visual, saccadic and memory signals are not identical, though strongly overlapping, indicating that the neuronal population codes are more complex than the sum of individual neuronal codes and are dynamically updated as a function of the ongoing behavior. We show that the neuronal population codes differ between spatial attention and spatial memory information, suggesting that these two functional processes are operated by distinct though overlapping neuronal populations. Last, we show that the neuronal population codes of spatial attention are very similar whether the cue is presented centrally or peripherally. These observations are discussed in the context of the spatial functions of the FEF.

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Poster

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Title: Reward strengthens the representation of task-relevant information in the prefrontal cortex during learning

Authors: ***B. MASSI**[1], C. H. DONAHUE[2], D. LEE[1];
[1]Neurosci., Yale Univ., New Haven, CT; [2]Gladstone Inst., UCSF, San Francisco, CA

Abstract: How an animal's behavior should be influenced by previous experience depends on environmental uncertainty. Previous studies have identified that neurons throughout the brain exhibit activity related to past events, but how environmental uncertainty affects these signals is unknown. To study this, we trained rhesus monkeys to perform a probabilistic reversal learning task in which the stability of the reward probabilities was manipulated. To initiate each trial, the

animals fixated a small square for 0.5s before two peripheral targets were presented. One target was associated with a high reward probability (80%), and the other with a low reward probability (20%). Following an additional 0.5s of fixation, a number of yellow dots surrounded the targets indicating the magnitude of potential reward available from each target. After a variable delay (0.5 ~ 1.2s), the animal was required to select one of the two targets. The stability of the reward probabilities was manipulated across blocks of trials. During volatile blocks, a red and green target were presented and underwent periodic uncued reversals in reward probability. During stable blocks, a cyan or diamond target always had an 80% reward probability, whereas an orange or square target was always associated with a 20% reward probability. Thus, the animals had to use recent outcome history to identify the target with the high reward probability in the volatile block, but not during the stable block. We analyzed single-unit activity from the dorsolateral prefrontal cortex (DLPFC), the orbitofrontal cortex (OFC), and the anterior cingulate cortex (ACC). In each region, we found that the outcome of the previous trial was more strongly represented in the volatile block than the stable block. Moreover, the current location of the target color chosen in the previous trial was most strongly represented in the DLPFC following rewarded trials in the volatile block. This indicates that reward strengthens the representation of task-related signals. Indeed, task-irrelevant signals related to the previously chosen location were present in the DLPFC, but unaffected by previous outcomes. We also found that signals related to the switch in target colors chosen in successive trials were present in all three regions and were also modulated by reward. Therefore, reward strengthens the representation of task-relevant information in the prefrontal cortex, providing a substrate for identifying important contingencies during reinforcement learning.

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Poster

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Title: Micro- and macro-circuit components of a putative attention filter

Authors: ***M. G. WHITE**, M. PANICKER, B. M. ROBERTS, B. N. MATHUR;
Pharmacol., Univ. of Maryland, Sch. of Med., Baltimore, MD

Abstract: The claustrum has broad cortical interconnectivity and transiently responds to salient stimuli. This enigmatic forebrain structure is therefore proposed to enable the intercortical communication necessary for attentional allocation (Mathur 2014). Using neuronal tract tracers we explore the connectivity profile of the mouse claustrum with cortical areas implicated in attention. We find that the claustrum displays dense interconnectivity with the anterior cingulate cortex (ACC) in particular, which is functionally implicated in attentional control in rodents. To examine the responsivity of claustrum neurons to ACC input, we use whole-cell patch clamp electrophysiology and optogenetics in acute mouse brain slices. We find that claustral spiny projection neurons faithfully fire in response to ACC afferent stimulation and that inhibitory claustral microcircuits provide feedforward and feedback inhibition, thus sculpting the ACC-mediated drive of claustrum projection neurons. Our results suggest that the claustrum is organized to filter and propagate incoming frontal cortical signals.

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Poster

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Title: Prefrontal neurons fulfill criteria for solving the credit assignment problem

Authors: ***W. F. ASAAD**[1], J. PERGE[2], E. N. ESKANDAR[3];
[1]Neurosurg., [2]Neurosci., Brown Univ., Providence, RI; [3]Neurosurg., Massachusetts Gen. Hosp. / Harvard Med. Sch., Boston, MA

Abstract: Credit assignment is the process by which a success or failure is linked with its potential cause. Proper credit assignment is necessary for any form of associative learning, but is more challenging when the causal environmental feature or stimulus is ephemeral and no longer

present when feedback is obtained about the outcome (temporal credit assignment problem) or when multiple potentially relevant features were concurrently present (structural credit assignment problem).
We trained two nonhuman primates to perform a learning task which entailed both a structural and a temporal credit assignment problem and recorded neuronal activity in the lateral prefrontal cortex. The task required the animals to learn which cue among four, presented earlier in a trial, was associated with a correct spatial choice at the end of the trial. We found that neuronal activity fulfilled the necessary criteria for providing a solution to the credit assignment problem: First, most neurons encoded the outcome of a trial, and many simultaneously encoded the identity of the relevant cue at the time of feedback, even though that cue was no longer present. This concurrent representation is necessary in order to link the outcome with the causal cue. Second, when the same neurons were recorded in a task with identical visual and motor elements, but with a different rule that rendered the identity of the cue irrelevant for learning, the cues were no longer represented in neuronal activity, demonstrating that the cue representation was actively engaged when necessary for learning.
Third, the neuronal representation of the cues at the time of feedback were sufficiently similar to the representation of the same cues at the time of their actual presentation, earlier in the trial, such that the identity of the correct cue could be determined from ensemble activity using a decoder trained at a different time point within the trial. This stability of representation over time is necessary for linking the outcome at the end of the trial with the causal feature that had appeared earlier.
Together, these data are consistent with the notion that neurons in the lateral prefrontal cortex provide the necessary, selective and stable representation of relevant features at the time of feedback to enable credit assignment.

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Poster

458. Attention and Frontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: Distractor suppression and distractor interference in the light of direct real-time access to the covert attentional spotlight from the frontal eye fields

Authors: *F. DI BELLO[1], S. BEN HADJ HASSEN[1], E. ASTRAND[2], S. BEN HAMED[1]; [1]Inst. Des Sci. Cognitives Marc Jeannerod, Bron Cedex, France; [2]Mälardalens Univ., Västerås, Sweden

Abstract: The frontal eye fields (FEF) plays a key role in top-down attentional control (Moore et al., 2003; Ekstrom et al., 2008, Ibos et al., 2013) and distractor suppression (Lennert et al., 2013, Suzuki et al., 2013). The inactivation of this brain area leads to a perturbation of visual attention processes which could be interpreted as deficits in attention shift commands (Wardak et al. 2006) and distractor suppression (Suzuki et al., 2013). Crucially, using classification methods applied to the ongoing time-resolved simultaneously recorded FEF multi-unit activity, we demonstrate direct two-dimensional (x,y) access to the spatial location of covert attention, i.e. to the attentional spotlight (Astrand et al., 2016). Surprisingly, this attentional spotlight is rarely anchored at the cued location, but moves around on the work screen. Here, we further demonstrate that overt behavior and distractor interference can only be interpreted if we take in account the precise location of this covert attentional spotlight at a given moment in the task. Specifically, we recorded bilateral neural activity from the frontal eye fields of two monkeys during a cued target detection task in which, in the half of the trials, a distractor was presented at a varying distance from the target. We reproduce the observation that the proportion of false alarm responses to a distractor decreases as the physical target-distractor distance increases. This is classically interpreted as an indication of a higher distractor interference effect at the cued location, where attention is assumed to be. We however show that this observation only holds when the attentional spotlight, as accessed from the FEF activity, is far away from both the distractor and the target. In contrast, if the attentional spotlight is either close to the target or to the distractor, distractor suppression is enhanced and fewer false alarms are produced. These observations are further supported by the fact that, at the single neuron level, distractor suppression increased as the distractor gets closer to the attentional spotlight. In summary, this work shows that the description of a central cognitive function, namely attention, remains flawed in the absence of a real-time access to its underlying covert processes as estimated from ongoing prefrontal neuronal activities and challenges our current understanding of distractor interference and suppression mechanisms.

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Poster

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Title: Mapping connections of prefrontal cortex using electrical microstimulation and fMRI in the macaque

Authors: *R. XU, N. P. BICHOT, P. K. WEIGAND, A. TAKAHASHI, R. DESIMONE; MIT, Cambridge, MA

Abstract: Lateral prefrontal cortex (LPFC) plays an important role in cognitive control. The architecture of LPFC remains unclear: is it comprised of distinct sub-systems with systematic differences in their functionality, or is it an integrated region in which different LPFC areas perform essentially the same computation? Determining the cortical connections of subregions of LPFC is invaluable as an anatomical constraint. Although previous tracer studies have looked at connections of LPFC, it is difficult to reach a clear and fine parcellation of this region due to the sparseness of injection sites and individual differences across animals. Moreover, the tracer results usually lack same-subject comparisons with neurophysiological observations in behaving animals. Here we mapped the connectivity of LPFC subregions *in vivo* using combined electrical microstimulation and fMRI (EM-fMRI) in the macaque.

We implanted a male macaque monkey (8 kg) with an oval-shaped chamber that covers areas anterior to the arcuate sulcus and along the principal sulcus. The animal was scanned in a 3T Siemens Trio magnet under propofol anesthesia (0.1-0.4 mg/kg/min) and with his eyes covered. We delivered 500 μA, 333 Hz charge-balanced bipolar current pulses for 210 ms every second in 30-sec blocks. The locations of stimulations sites were confirmed with T1 and T2 anatomical MRI images during each session, before and after microstimulation. At each site, we collected four runs (34 mins) of MION signals using an EPI sequence (2 mm isotropic), to balance between maximizing SNR and minimizing scanning time.

We stimulated LPFC sites close to the junction between the arcuate and principal sulci, including the frontal eye field (FEF), area 46, dorsolateral prefrontal cortex (DLPFC), and the ventral preacuate (VPA) region we recently found to play an important role in feature-based visual attention (Bichot et al., 2015). The EM-fMRI connections were in general consistent with monosynaptic connections reported in previous tracer studies. Based on the preliminary data, we found differences between the connections of ventral and dorsal regions relative to the principal sulcus. The connections of ventral sites (in VPA, ventral bank of area 46, and part of FEF) included most ventrolateral PFC (VLPFC) and few DLPFC regions. On the other hand, connections of dorsal sites (in DLPFC, dorsal bank of area 46, and part of FEF) included most DLPFC and few VLPFC regions. Most interestingly, only the ventral sites seemed strongly connected to mid-to-high visual areas and lateral intraparietal cortex.

Disclosures: R. Xu: None. **N.P. Bichot:** None. **P.K. Weigand:** None. **A. Takahashi:** None. **R. Desimone:** None.

Poster

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Title: Effects of attention on neural activity of orbitofrontal cortex

Authors: *Y. XIE, C. NIE, T. YANG;
Inst. of Neurosci., Shanghai, China

Abstract: Attention can influence the responses of individual neurons throughout visual cortex. It is unknown if it also affects the responses of neurons encoding more abstract features associated with visual stimuli. Neurons in the orbitofrontal cortex (OFC) are found to encode value associated with visual stimuli. Here we investigated how visual attention may affect their responses. Two monkeys were trained to perform a passive viewing task in which they had to maintain their fixations when simple shapes were presented peripherally on a computer screen. The shapes were randomly selected from a pool of five, each associated with 0, 1, 2, 4, and 8 drops of water, respectively. When shapes were presented alone in a trial, their associated reward was delivered at the end of the trial. When two shapes were presented together, one of them was randomly selected and its associated reward was delivered. We measured monkeys' pupil dilation responses to the shapes and confirmed that they understood the value of each shape. When two shapes were presented at the same time, the dilation of pupil size mostly reflected the value of the shape that predicted a larger reward. We next recorded single unit activity in OFC. As expected, a subset of neurons encoded shape's value during the shape presentation period. When two shapes were presented together, their responses could be explained by the weight sum of their responses to the single shapes. The weight for the response to the higher valued shape dictated the neurons' responses. Next, we manipulated monkeys' attention by temporarily rotating one of the shapes for 100 ms in some two-shape trials. The rotation was not linked to the reward. We again modeled the neurons' responses with the weighted sum model and compared the weights against the weights obtained from the no-rotation trials. We found that the weight for the responses to the rotated shape increased. The latency for this weight increase was 77 ms in one monkey and 177 ms in the other. Behavior analysis suggested that the difference in the latency in two monkeys may be explained by a combination of both bottom-up and top-down attention mechanisms. Our results demonstrate how attention may affect OFC neural activities.

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Poster

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Title: Neuronal modulations in prefrontal cortex are associated with multiple components of visuospatial attention

Authors: *T. Z. LUO, J. H. R. MAUNSELL;
The Univ. of Chicago, Chicago, IL

Abstract: Neuronal signals related to visuospatial attention are found in widespread brain regions, and these signals are generally assumed to participate in a common mechanism of attention. However, the effects of attention on target detection can be separated into two distinct components: spatially selective shifts in either the response criterion or the perceptual sensitivity of the observer. We have previously found (Luo & Maunsell 2015) that when monkeys are trained to do a variant of the Posner attention paradigm, a task used in many single-neuron studies of attention, enhanced performance is typically associated with changes in both criterion and sensitivity. But when these two components of attention were dissociated using a specifically designed task, we found that attention-related neuronal modulations in area V4 of visual cortex corresponded to behavioral shifts in sensitivity, but not shifts in criterion.
More recently, we have been examining visual responses in prefrontal cortex (areas 45 and 46). Neurons with spatially selective visual responses were selected using a memory-guided saccade task. In contrast to responses in V4, visual responses in prefrontal cortex were modulated by behavioral changes in both criterion and sensitivity. Both increases in sensitivity (enhanced discrimination) and decreases in criterion (greater tendency to respond) increase the response of visual neurons in prefrontal cortex. During the visual stimulus period, increases in sensitivity were associated with an average of 61% increase in firing rates, and decreases in criterion were associated with a 23% increase.
These results show that attention-related neuronal modulations across prefrontal cortex and visual cortex are not associated with a single mechanism of visuospatial attention. Instead, prefrontal cortex contributes to multiple mechanisms of attention, while visual cortex is associated with one of these mechanisms. The results indicate that visuospatial attention is not a unitary process in the brain but instead consists of separable mechanisms.
Luo, T.Z., Maunsell, J.H.R., 2015. Neuronal modulations in visual cortex are associated with only one of multiple components of attention. Neuron 86, 1182-1188.

Disclosures: **T.Z. Luo:** None. **J.H.R. Maunsell:** None.

Poster

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Title: Circuit mechanisms of prefrontal contribution to visual behavior

Authors: ***R. HUDA**[1], G. PHO[1], L. GUNTER[1], I. WICKERSHAM[2], M. SUR[1];
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Abstract: A fundamental building block of voluntary behavior is the ability to respond to information from the environment with appropriate motor actions. While seemingly simple, this process of transforming sensory information into motor commands consists of at least two distinct steps - detecting sensory information relevant to current behavioral goals from a noisy environment and selecting appropriate motor actions based on those stimuli. While neural substrates of sensorimotor mapping and attention have been widely studied in non-human primates, many questions remain due to lack of tools that can selectively probe and manipulate neural activity, especially in a projection-specific manner. The availability of optogenetic and viral-based gene expression tools in mice enables the interrogation of cell type-specific contributions to circuit-level mechanisms underlying these cognitive phenomenon. Here, we devised a visual sustained-attention, sensorimotor task for head-fixed mice to probe the selective contribution of specific neuronal subsets. First, we used rabies viruses to identify a caudal midline prefrontal region (anterior cingulate cortex) that is anatomically poised to contribute to both attentional processing of visual stimuli, owing to its top-down projections to visual cortex,

and sensorimotor transformation, due to its feed-forward projections to superior colliculus. Mice were trained to report the spatial location of a visual stimulus by rotating a ball. We made the task attention-demanding by including a variable foreperiod that introduces uncertainty in temporal expectancy for visual stimulus onset. In the first set of experiments, we found that optogenetic inactivation of this prefrontal area compromises performance on the task. Subsequently, two-photon microscopy and projection-specific optogenetic inactivation has allowed us to probe the functional contribution of visual cortex- and superior colliculus-projecting prefrontal neurons to sustained attention and sensorimotor transformation, respectively.

Disclosures: **R. Huda:** None. **G. Pho:** None. **L. Gunter:** None. **I. Wickersham:** None. **M. Sur:** None.

Poster

458. Attention and Frontal Cortex

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 458.13/KKK43

Topic: H.01. Animal Cognition and Behavior

Title: Comparison of the contribution from superior colliculus and frontal eye field to covert spatial attention

Authors: *A. BOLLIMUNTA, A. R. BOGADHI, R. J. KRAUZLIS;
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Abstract: Both superior colliculus (SC) and frontal eye fields are known to contribute to covert spatial attention. Comparing their contributions by measuring the deficits caused by reversible inactivation (e.g., muscimol) is confounded by the differences in the amount of the pharmacological agent required to cause the deficit, and by differences in the size and organization of these two brain areas. Here, taking advantage of the fact that reversible inactivation also causes changes in metrics of overtly generated saccades, we measured the contribution of SC and FEF to covert spatial attention relative to their contribution to saccades. Two male macaque monkeys were trained on a visually guided saccade task and a spatial attention task. The attention task consisted of two types of trials: Foveal attention (FA) and Peripheral attention (PA). During PA trials monkeys had to maintain fixation and detect a change in the direction of motion stimulus presented in the periphery. During FA trials the motion stimulus should be ignored and monkeys had to detect changes in the luminance of the central fixation point. Blocks of FA and PA trials were interleaved. The magnitude of change in PA and FA was set at threshold level. Monkeys reported the relevant event in each type of trial by

releasing a lever to get a juice reward. Reversible inactivation of SC (n = 33) and FEF (n = 19) were done by injecting muscimol. The amount of muscimol injected ranged from 0.3 to 0.5 µl in SC and 1.5 to 3.0 µl in FEF. The efficacy of inactivation in each session was gauged by measuring the increase in latency of visually guided saccades to targets placed at the same location as the peripheral stimulus in the attention task. Performance in the attention task was measured both before and during inactivation.
The deficit in the visually guided saccade task was used as a benchmark to compare the deficit in the attention task. The increase in saccade latencies ranged from 6 to 115 ms in SC, and 28 to 140 ms in FEF. A significant deficit in the attention task was observed in 78% of SC inactivation sessions and in 26% of FEF sessions. The probability of observing a deficit in the attention task given a saccade latency deficit was estimated using the saccade latencies as predictive variables in logistic regression analysis. Results show that the probability of observing a deficit in the attention task during FEF inactivation required a saccade deficit that was about twice as large as during SC inactivation. Hence, relative to saccades, covert spatial attention appears to be more dependent on activity from the SC than from FEF.

Disclosures: **A. Bollimunta:** None. **A.R. Bogadhi:** None. **R.J. Krauzlis:** None.

Poster

458. Attention and Frontal Cortex

Location: Halls B-H

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Program#/Poster#: 458.14/KKK44

Topic: H.01. Animal Cognition and Behavior

Title: Prefrontal inter- and intra-hemispheric neuronal noise correlations depend on the ongoing behavior.

Authors: *S. BEN HADJHASSEN, E. ASTRAND, C. WARDAK, S. BEN HAMED;
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Abstract: The frontal eye field (FEF) is a brain area involved in the control of eye movements and attention. Importantly, it is a crucial node of the default-mode network (Vincent et al., 2007), the activity of which varies as a function of the arousal and attentional state of the subject. However, the exact neuronal processes underlying the characteristic default-mode network connectivity and task-dependence is still unclear. Here, we focus onto inter- and intra-hemispheric FEF neuronal noise correlations, taken as a possible neurophysiological correlate of the functional connectivity obtained with fMRI, and we investigate how these vary as a function of the ongoing behavior. In a recent study (Astrand et al.,2016), we show that these noise correlations are significantly lower on correct trials than on incorrect trials. Increased noise

correlations can be seen from trial onset to trial end, independently from any attentional orientation, and they correlate with a degraded processing of both sensory and cognitive information. In the present study, we provide evidence to the effect that noise-correlations do not only depend on overt behavioral performance, but are dynamically tuned to the ongoing behavioral requirements of the task. To this end, we performed bilateral simultaneous multiunit activity (MUA) recordings from the right and the left FEF of two macaque monkeys, using two 24-contact multi-electrodes, while the monkeys were engaged in different types of simple tasks. In order to quantify the impact of the ongoing behavior onto the inter-and intra-hemispheric noise correlations, we calculated for specific timings in each task, four different parameters:trial-to-trial noise correlations, mean firing rates, the standard deviation around the firing rates as well as the associated Fano factor. Our analyses demonstrate a clear dependence of the inter- and intra-hemispheric noise correlations on the ongoing task as well as on the ongoing overt performance. Importantly, this change in noise correlations is independent of changes in the related overall firing rate properties. Importantly, we describe task-specific topographically organized patterns of inter-hemispheric noise correlations changes. We propose that inter- and intra-hemispheric noise correlations in the FEF dynamically adjust sensory and cognitive processes to the ongoing motivational and behavioral requirements of the task

Disclosures: **S. Ben Hadjhassen:** None. **E. Astrand:** None. **C. Wardak:** None. **S. Ben Hamed:** None.

Poster

458. Attention and Frontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR

NSERC

EJLB Foundation

Title: Neural network properties are dynamically modulated by attention in primate lateral prefrontal cortex

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Abstract: Recent studies have used graph theoretical approaches to analyze complex network activity in the brain. One seemingly ubiquitous finding is the existence of small-world network from the microscopic to macroscopic scale. These networks are characterized by nodes which are highly clustered locally, and complemented by short path lengths which globally connect each node in the network. We aimed to explore the network properties of *in vivo* neural ensembles in the prefrontal cortex, and how these networks are affected by top-down attention. We implanted multi-electrode arrays in the lateral prefrontal cortex (lPFC) of two non-human primates (*Macaca Fascicularis*) and recorded spiking activity from neurons during a task that required the animals to covertly attend towards one of four spatial targets on a computer screen. Using firing rates computed for each neuron in non-overlapping bins during each trial, we obtained Pearson's correlation matrices between units for different time points during a trial. Significant correlations ($P<0.05$) in each matrix were used to compute clustering coefficients and average path lengths for each unit. Small-world network properties were quantified using a double ratio, with the numerator being the empirical clustering coefficient divided by that of a random network, and the denominator being the observed path length divided by that of a random network. The significance of this small-world network index was assessed using a permutation test. Interestingly, we found that the small-world index increased when monkeys allocated spatial attention. We have previously shown that ensembles of neurons in this area can be used to robustly decode where in space a monkey is allocating its attention. Therefore it is possible that these dynamic network properties enable the optimal encoding of spatial information during attention.

Disclosures: **L. Duong:** None. **M. Abass:** None. **A. Sachs:** None. **J. Martinez-Trujillo:** None.

Poster

458. Attention and Frontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01EY019273-01

Title: Responses of frontal eye field neurons in a visual foraging task

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Abstract: To find an object effectively during visual search, we use strategies to look at the most probable places and to avoid unlikely places or objects that have already been looked at. The priority map hypothesis is one of the theories that explains how these strategies are formed at the neural level. We have previously shown that activity in the lateral intraparietal area (LIP) acts like a priority map, in which both pre-attentive bottom-up signals and top-down cognitive feedback are integrated to represent the attentional priority of stimuli in the visual field. This is then used to guide eye movements. Although the activity in LIP represents the behavioral importance of objects in visual field, it does not correlate directly with eye movement behavior. We previously proposed that a single additional step of divisive normalization is necessary before the information present in LIP can be used to guide eye movements and hypothesized that this occurs as information is fed forward to the Frontal Eye Field (FEF). To test this hypothesis, we recorded the responses of FEF neurons in two animals while they performed a visual foraging task. The animals searched through 5 potential targets (Ts) and 5 distractors to find a target loaded with reward. After the stimuli appeared, the animals were free to move their eyes. Stimuli were spaced such that when the animal was looking at one stimulus, another was in the FEF neuron's response field. FEF neurons that contributed to the foraging task could be placed in four functional categories: 1) Neurons that showed an increase in response to previously fixated Ts. 2) Neurons that represented the attentional priority of the visual scene in a similar way to LIP. 3) Movement neurons, which didn't differentiate the attentional priority of the objects but responded to the initiation of an eye movement. 4) Neurons that showed a late response that correlated with predicted value, consistent with downstream normalization from LIP. Many of these neurons started off responding more to previously fixated Ts, but changed their responses about 150 ms after fixation onset, a time that is consistent with feedforward normalization. We conclude that the guidance of eye movements is driven by a network in which processing occurs at each step. LIP responses, the gain of which varies as a function of set size, are biased by low-level salience and top-down cognitive feedback. We propose that post-normalized FEF responses incorporate subjective value and, we predict, will not show set size effects, allowing the decision of where to look to be made with an absolute threshold. The output of this process is represented in the responses of the movement neurons.

Disclosures: **K. Mirpour:** None. **Z. Bolandnazar:** None. **J.W. Bisley:** None.

Poster

458. Attention and Frontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant Number 15H01673

Title: Animal model of spatial neglect in macaque monkeys

Authors: *K. TSUJIMOTO[1,3], M. SAWADA[1], M. FUKUNAGA[2], M. YOSHIDA[1,3];
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Abstract: Spatial neglect is a characteristic failure to explore the side of space contralateral to a brain lesion, which cannot be explained by primary sensory or motor disorders. The purpose of this study is to establish monkey model of spatial neglect. The spatial neglect of the human involves the dorsal attention network and the ventral attention network. Anatomical studies suggest that the homologous region of the ventral attention network in the human includes superior temporal gyrus (STG) in monkeys. Based on this findings, we made a lesion in the right STG of three monkeys and evaluated behavior of the monkeys after the lesion. To evaluate their behavior in the cage, we used a 'food-choice' task. In this task, a piece of apple was hidden in a well under a cover with a stripe pattern. If the monkeys selected the well with a striped pattern among the wells without patterns, the monkeys got a reward. In this task, reaction time to touch the well in the contralesional side was longer than that in the ipsilesional side for 3 month after the lesion. To evaluate their behavior on the monkey chair, we used a 'target-selection' task and a free-viewing task. The visual stimuli of the target-selection task was presented on a display with a touch panel. The visual stimuli consists of a target and nine distractors. If the monkeys touched the target within 2 sec, the monkeys got a reward. In the target-selection task, the percentage of correct response to the targets in the contralesional side was lower than that in the ipsilesional side for one month after the lesion. In the free-viewing task, fifty-six natural images in total were used as test images and were randomly presented. In the free-viewing task, gaze positions was strongly biased toward the ipsilesional side for more than one month after the lesion. Motor deficit and sensory deficit were not detected in the behavior on the cage and the chair. These results suggest that the STG lesion induced spatial neglect in the monkeys. We also evaluated the functional connectivity of the monkeys before and after the lesion using resting-state fMRI. For this purpose, we measured the BOLD activity of the monkeys under isoflurane anesthesia (1%) with a 3T MRI before 1week, and 1, 2, 3, 4 weeks after the lesion. The analysis suggests that the functional connectivity between the posterior parietal cortex and the prefrontal cortex was decreased for one week after the lesion. Since this result is consistent with findings with human spatial neglect patients, our results suggests that the neural mechanisms of behavioral deficits in monkeys are similar to those of human.

Disclosures: K. Tsujimoto: None. **M. Sawada:** None. **M. Fukunaga:** None. **M. Yoshida:** None.

Poster

459. Attention in Visual Cortical Areas

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Support: NIH/NIDA NRSA F31DA037651

NIH/NIDA R01 DA017960

PhRMA Foundation Pre-Doctoral Award

Title: Methylphenidate enhances early stage sensory signal processing within the rat during performance of a visual signal detection task

Authors: *R. L. NAVARRA[1], B. D. CLARK[2], B. D. WATERHOUSE[3];
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Abstract: Abnormal processing of sensory information is a core feature of many neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD). The psychostimulant, methylphenidate (MPH), is used clinically to treat ADHD as well as off-label as a performance enhancing drug (PED) by healthy individuals. MPH enhances catecholamine transmission via blockade of norepinephrine and dopamine reuptake transporters. However, it is not clear how these effects impact neural circuits responsible for sensory signal processing and behavioral outcomes. We have found that MPH increases both speed and strength of individual and ensemble neuronal responses to visual stimuli within the dorsal lateral geniculate nucleus (dLGN) of the rat while improving the speed to make correct responses during performance of a visual signal detection task, suggesting that enhanced sensory signal transmission may be a significant component of the action of PEDs. Here, we investigated the effects of MPH on an additional measure of early stage sensory signal processing, the visual evoked potential (VEP). VEPs are waveforms produced in response to visual stimuli that can be extracted from local field potentials within the dLGN. MPH (2 mg/kg, i.p.) reduced the latency and amplitude of P30, i.e. first positive deflection of the VEP occurring 30 msec following stimulus presentation. It is generally accepted that P30 represents first prominent indication of signal transmission within the dLGN (Meeren et al., 1998). A reduction in P30 latency suggests visual signals during task performance are being transmitted to the dLGN more quickly following MPH administration. MPH also decreased the amplitude of the P30 response. Similar effects were observed in the visual cortex of monkeys performing a cue targeting task (Sundberg et al., 2012), where selective attention decreased both the latency and the magnitude of the first deflection of the VEP to target cues. Thus, it appears MPH effects on dLGN VEP's mimic top-down influences of selective

attention on VEP's recorded from monkey visual cortex. These results are consistent with faster sensory signal processing and better visual signal detection. Furthermore, in the present study immunostaining showed that catecholaminergic innervation to the dLGN is solely noradrenergic. This work suggests that MPH, acting via noradrenergic mechanisms, can substantially impact early stage sensory signal processing and subsequent behavioral outcomes. As such, sensory enhancement may be a significant component of psychostimulant-induced performance enhancement in ADHD patients and healthy individuals.

Disclosures: R.L. Navarra: None. **B.D. Clark:** None. **B.D. Waterhouse:** None.

Poster

459. Attention in Visual Cortical Areas

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NIDA DA017960

Title: Indvidual rats choose alternate, sub-optimal strategies in a (flawed) signal detection task. An observational study, with a moral.

Authors: *B. D. CLARK[1], J. S. SHUMSKY[1], B. D. WATERHOUSE[2];
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Abstract: While reviewing records of rats that had performed a signal detection task over several weeks - months, we observed instances of high-scoring individuals reverting, abruptly and permanently, to lower levels of baseline performance. Examining the sequence of trials, we discovered that these animals had switched, in part, to a win-shift, lose-stay strategy. which, due to the design of the task, was rewarded in roughly 58% of trials. This departure from desired randomness in trial type presentation order emerged from the use of a sampling-without-replacement scheme to select trial type in the controlling software. While the use of this scheme prevents drastic imbalance in trial-type totals, it does so at the cost of introducing modest predictability: once a trial's type is given (signal vs non-signal), the next trial is slightly more likely than chance to be of the other type. At a point, the animals we observed shifted to this easier but less-rewarded strategy, and thereafter they used it, accepting a lower reward rate than they could have achieved by attending to the signal light. Additional records were discovered of mid-level performers who had been using win-shift behavior throughout training. Interestingly, the animals that employed this method (~15% of tested rats) appeared to use a hybrid approach

depending on intertrial delay: for short delays, the win-shift strategy was prominent, but for the longest delays, animals used the signal detection strategy. The transition between these typically appeared at intertrial delays of 6-8 seconds. Our task design may inadvertently resemble a cognitive effort task (Cocker et al., 2012), with the easier, win-shift, lose-stay approach being adopted by "slacker" individuals. The moral: Departing from true randomness in study design can create opportunities for subjects to behave in unexpected ways.
Cocker PJ, Hosking JG, Benoit J, Winstanley CA (2012) Sensitivity to cognitive effort mediates psychostimulant effects on a novel rodent cost/benefit decision-making task. Neuropsychopharmacol 37: 1825-1837.

Disclosures: **B.D. Clark:** None. **J.S. Shumsky:** None. **B.D. Waterhouse:** None.

Poster

459. Attention in Visual Cortical Areas

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RPB Career Development Award

Title: Population encoding of attentional states in the absence of visual stimulation

Authors: *A. C. SNYDER[1,3,5], B. M. YU.[3,4,5], M. A. SMITH[1,5,2,6];
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Abstract: The visual world contains more information than the brain can process. Through selective attention we preferentially process relevant information at the expense of ignored items.

These processing improvements are reflected by increased firing rates and decreased response (co)-variability during visual stimulation. However, the preparatory state changes that precede and underlie these processing differences remain mysterious. In particular, evidence to date suggests attention minimally influences baseline firing rates before the stimulus is presented. In other words, the spatial locus of attention would be difficult or impossible to decode from single-neuron activity before the stimulus is presented, despite the anticipatory nature of attention. We used high-yield population recordings to test the hypothesis that anticipatory attention states are reflected in characteristic patterns of activity across the population, without a change in average population firing rate. We found, consistent with prior reports, that attention increased sustained firing rates in response to attended visual stimuli in macaque V4 neurons, but did not affect average baseline rates. When analyzing baseline activity patterns using a high-dimensional state-space approach, we found that V4 populations reliably encoded the attention state during the baseline with characteristic patterns (but not overall rates) of activity. We simultaneously recorded population activity in dorsolateral prefrontal cortex (PFC), reasoning this area implicated in executive functions might guide V4 attention states. We tested the hypothesis that the attention state as decoded from baseline PFC population activity (in high-dimensional space) predicts the response of a V4 population to a subsequent stimulus. Our results strongly support the encoding of attention state by patterns of population activity that are not visible at the level of individual neurons or pairs. Furthermore, our simultaneous recordings suggest that feedback from prefrontal cortex has an important role in generating the attention state in visual cortex.

Disclosures: **A.C. Snyder:** None. **B.M. Yu.:** None. **M.A. Smith:** None.

Poster

459. Attention in Visual Cortical Areas

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01EY005911

Title: Neuronal correlates of attentional selectivity in area V4 is independent of motivational context

Authors: *S. GHOSH, J. H. R. MAUNSELL;
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Abstract: Previous work from our lab has shown that when monkeys shift their attention to increase their behavioral sensitivity, i.e, an ability to detect a stimulus, at one visual field

location, neurons in area V4 with receptive fields in that location respond more strongly (*Luo and Maunsell, 2015*). However, in those experiments an increase in sensitivity in one hemifield was always associated with a decrease in sensitivity in the other hemifield. The observed modulation of neuronal activity could therefore depend on the absolute sensitivity at the receptive field location regardless of it's value at distant site, or it could depend on the relative sensitivity at the two locations (attentional selectivity). We therefore trained two rhesus monkeys to do an orientation change detection task in which we could keep their behavioral sensitivity constant at the receptive field location while varying at a distant location over a broad range. If neuronal responses depend only on sensitivity and not on selectivity, this manipulation would not affect their responses. Using chronically implanted multielectrode arrays, we recorded spikes from populations of neurons in area V4. We found that varying sensitivity at the distant site substantially modulated V4 responses. As the behavioral sensitivity decreased at the distant location away from receptive fields, neuronal responses became stronger, despite the fact that the animal's overall performance declined. A sensitivity change at the distant site was about half as effective in changing the firing rate of the neurons as the same change in sensitivity at the receptive field location. Thus, the responses of V4 neurons are increased not only by increases in behavioral sensitivity at the receptive field location, but also by increases in attentional selectivity for the receptive field location relative to a distant location. Changes in sensitivity and selectivity could be controlled by adjusting either the relative reward sizes associated with stimuli at the two sites, or the relative difficult of the orientation changes at the two sites. The effects of sensitivity and selectivity were the same regardless of how the changes were motivated.

Disclosures: S. Ghosh: None. **J.H.R. Maunsell:** None.

Poster

459. Attention in Visual Cortical Areas

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Topic: H.01. Animal Cognition and Behavior

Support: NWO-MGW 406 – 12 – 072

Title: Dual-task interference in macaque early visual cortex

Authors: *J. POSSEL[1], M. W. SELF[1], P. R. ROELFSEMA[1,2,3];
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Abstract: Multitasking generally leads to suboptimal performance (for example slowing of responses and/ or more mistakes). Task interference occurs when two tasks have to be performed at the same time or with a short stimulus onset asynchrony (SOA). Responses are typically slower to the second task if the SOA is within the interference range. This effect is known as the psychological refractory period (PRP). There are numerous theories that speculate on why it is not possible to perform two tasks in parallel. Some studies suggest that the sharing of a common resource between two tasks functions as a processing bottleneck and therefore causes the delayed response to the second task. Others suggest that the selection of two responses cannot happen in parallel. However, it is not clear which neural mechanisms underlie this behavioral phenomenon.
We studied how the PRP influences neural activity in different areas along the visual hierarchy (i.e. V1 and V4) in monkeys using implanted multi-electrode arrays. Task 1 was a spatial location judgment which the monkeys reported via a hand movement. Task 2 was a curve-tracing task in which the monkey mentally traced a target curve and ignored a distracter curve and made a saccade to a circle at the end of the target curve. We examined the influence of dual-task interference on the early sensory responses and the later attentional selection signals.
We found that monkeys also show a PRP effect as their RTs in the curve-tracing task (task 2) were longer at short SOAs. Previous studies have revealed attentional modulation of neuronal responses in V1 and V4 in the curve-tracing task. We found that the PRP reduced the attentional modulation in V1 and V4 and that it also increased the latency of the attentional modulation in V1. The PRP had comparatively little influence on the initial visually driven activity. We conclude that the PRP is associated with a decrease in attentional selection that is required to solve task 2. Our results thereby support theories that propose that there is a limitation of attentional processing (shared resource models) if two tasks need to be performed in close succession.

Disclosures: **J. Possel:** None. **M.W. Self:** None. **P.R. Roelfsema:** None.

Poster

459. Attention in Visual Cortical Areas

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Program#/Poster#: 459.06/KKK53

Topic: H.01. Animal Cognition and Behavior

Title: Spatial summation sub-compartments in the orientation column

Authors: *X. SONG[1], M. LI[2], T. XU[3], D. W. HU[2], A. W. ROE[1], C. Y. LI[2,3];
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Abstract: In the primary visual cortex (V1) of cats, neurons with different orientation preferences are arranged in a pinwheel-like structure with a number of iso-orientation domains (IODs) around a pinwheel center (PC). We previously found that there are clusters of neurons in V1 organized according to their spatial summation properties (surround suppressive or non-suppressive, Neuron 35:547-553, 2002). However, the spatial relationship between these two functional architectures remains unclear. In this study, using intrinsic signal optical imaging combined with local field potential (LFP) and single-unit recording methods, we found three concentric sub-regions centered on the orientation-pinwheel. The central most region contained neurons with small receptive fields and strong suppressive surrounds, while the outermost region contained neurons with larger receptive fields and weak suppressive surrounds. We hypothesize that orientation pinwheels s and surround suppression zones are orthogonally represented parameters in cat V1. These inter-related organizations may be significant for further understanding the functional organization of visual cortex and provide new bases for visual features integration.

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Poster

459. Attention in Visual Cortical Areas

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Support: NSF GRFP

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PNI Innovation Award

Title: Communication between the pulvinar and the layers of Area V4 during selective visual attention

Authors: *R. LY[1], S. KASTNER[1,2];
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Abstract: A fundamental question in neuroscience is how large-scale networks of neurons interact to give rise to cognitive function. A recent study (Saalmann et al., 2012) suggests that the pulvinar nucleus of the thalamus influences cortical visual areas during selective visual attention in the alpha (8-15 Hz) frequency band. The laminar profile of this influence could inform network-level models of visual processing and attention, but is as yet unknown.
We simultaneously recorded laminar activity in area V4 using a linear multielectrode array, and spiking activity and local field potentials in the pulvinar using single-unit microelectrodes in a macaque monkey while the animal performed a spatial attention task. Receptive fields (RFs) overlapped across V4 layers and the pulvinar, suggesting that we recorded from an interconnected network. We computed oscillatory power and coherence within area and layer, and Granger-causal influences between the pulvinar and laminar currents in V4, at gamma and alpha/beta frequencies. We compared these measures when the animal attended to the shared RF versus when the animal attended away and when there was a stimulus in the RF versus when there was not. These effects are discussed in the context of recent evidence demonstrating gamma synchrony and Granger-causality across areas as markers of bottom-up processing and alpha/beta synchrony and Granger-causality across areas as markers of top-down processing.

Disclosures: R. Ly: None. **S. Kastner:** None.

Poster

459. Attention in Visual Cortical Areas

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

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Title: The attentional bias for novelty fluctuates across time

Authors: *D. VATTEROTT, J. GOTTLIEB;
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Abstract: A common postulate of learning theories is that animals by default assign high salience to novel items, facilitating their ability to detect, attend to, and learn about these items (Brockmole & Henderson, 2005; Foley et al., 2014). However, psychophysical studies have produced mixed results documenting either novelty or familiarity preferences in children and adults (Hunter & Ames, 1988; Park, Shimojo, & Shimojo, 2010), suggesting that novelty biases are not fixed but depend on the task or context. To date, little is known about this dependence, and especially the role of the subjects' behavioral state. To investigate this question we designed a task where rhesus monkeys (Macaca mulatta) could express their spontaneous novelty preference during a free-viewing epoch coinciding with the inter-trial intervals of a fixation task. Two monkeys completed behavioral trials by simply fixating a central point for a juice reward. Following reward delivery, the monkeys were presented groups of 4 images (colored fractal patterns), the fixation point was extinguished and the monkeys were allowed to freely view the fractal patterns. The images remained on the screen for a 2 second period until they were extinguished and the next trial started. Groups of 4 fractals were randomly selected and presented over several daily sessions (several hundreds of trials), familiarizing the monkeys with the individual images and their contextual grouping. In 20% of trials, a randomly selected familiar image was replaced by a novel, trial-unique item. Both monkeys showed a novelty bias, fixating the novel items more frequently than they fixated the familiar ones, and this bias grew as the monkey gained exposure to the familiar patterns. Importantly, the bias was not constant but showed slow fluctuations, waxing or waning over stretches of ~100 trials during a session. The fluctuations were independent of the visual salience of the novel patterns (measured with a canonical salience model (Walther & Koch, 2006) and were also independent of fluctuations in instrumental performance, which had a shorter time scale. The results suggest that this paradigm can reliably elicit state-dependent novelty biases, paving the way to investigating their neuronal correlates.

Disclosures: **D. Vatterott:** None. **J. Gottlieb:** None.

Poster

459. Attention in Visual Cortical Areas

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Program#/Poster#: 459.09/KKK56

Topic: H.01. Animal Cognition and Behavior

Title: Superior colliculus inactivation with functional imaging reveals novel nodes in the control of spatial attention

Authors: *A. R. BOGADHI[1], A. BOLLIMUNTA[1], D. A. LEOPOLD[2], R. J. KRAUZLIS[1]; [1]Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: We recently showed that inactivation of the superior colliculus (SC) leads to attention deficits in a behavioral task despite normal attention-related neuronal modulation in visual cortex (Zenon & Krauzlis, 2012). This finding suggests that SC inactivation might affect brain regions downstream to visual cortex, leading to the observed spatial attention deficits. To identify these potential brain regions, we employed fMRI with and without SC inactivation in two monkeys trained to perform a spatial attention task in a vertical scanner.

The stimulus sequence on each imaging run (~480s) consisted of three different blocks: Baseline (B), Foveal Attention (FA) and Peripheral Attention (PA) blocks. B blocks (~10s long) were interleaved between FA and PA blocks, each 20s long. In B block trials, the relevant stimulus was a central fixation point that dimmed at randomized times. FA block trials were similar to B 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 maintain central fixation and report the relevant stimulus change (fixation dimming in B & FA blocks, peripheral motion change in PA blocks) by releasing a lever to get a juice reward.

A total of 225 runs from 16 control sessions and 220 runs from 18 inactivation sessions in 2 monkeys were included. Eye movements were recorded during each imaging run. For our preliminary analysis, we identified several cortical (MT,FST,LIP,FEF) and sub-cortical (SC,Caudate,Pulvinar) clusters in the control dataset that showed attention-related modulation by contrasting PA and FA conditions (t-scores > 3.39). The effect of SC inactivation on BOLD responses in these clusters was quantified as a t-score comparing the attention-related increase in BOLD during control and inactivation.

During SC inactivation, the monkey's behavioral performance was significantly impaired for stimulus changes in the contralateral hemifield. During the same blocks, we observed a significant drop in the attention-related BOLD modulation in fronto-parietal areas implicated in the control of spatial attention. However, the biggest reduction in the attention-related BOLD modulation was observed in area FST in the superior temporal sulcus. At the same time, several sub-cortical areas (pulvinar, caudate) showed a significant reduction in attentional modulation on the side of the injection. These results identify area FST as a potentially important node in the cortical and sub-cortical network for the control of spatial attention.

Disclosures: A.R. Bogadhi: None. **A. Bollimunta:** None. **D.A. Leopold:** None. **R.J. Krauzlis:** None.

Poster

459. Attention in Visual Cortical Areas

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Support: Human Systems Technologies for Future Air Force Challenges, Program I: Neuroscience and Medical Imaging. U.S. Air Force Research Laboratory, BAA-PKD-08-0006

Title: How configural is the configural superiority effect? a neuroimaging investigation of configurality in visual cortex

Authors: O. M. FOX, *A. HAREL, K. B. BENNETT;
Psychology, Wright State Univ., Dayton, OH

Abstract: Many regions in occipito-temporal cortex (OTC) are sensitive to the organization of elements in the visual field. When stimulus features are perceptually well-organized, one often observes an improvement in behavioral performance, a phenomenon known as the Configural Superiority Effect (CSE). Prior neuroimaging work on CSE regards it as an "all or none" phenomenon, focusing on the contrast between configural and non-configural stimuli. However, it is still not clear whether CSE emerges also in response to deviations from these two endpoints. The current study examined the extent to which behavioral and neuroimaging markers of CSE are responsive to the degree of configurality in visual displays. Subjects were tasked with reporting the anomalous quadrant in a visual search task while being scanned. Degree of configurality was manipulated by incrementally varying the rotational angle of the features within the stimulus arrays. Behaviorally, we observed faster response times with increasing levels of configurality. These behavioral changes were accompanied by increases in response magnitude across multiple visual areas in OTC, including early visual cortex as well as object-selective cortex. Our findings suggest that the neural correlates of CSE can be observed even in the absence of "perfect" configurations, and demonstrate that configural information is already present at early stages of the visual hierarchy.

Disclosures: **O.M. Fox:** None. **A. Harel:** None. **K.B. Bennett:** None.

Poster

460. Executive Function: Models of Disorders

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Topic: H.01. Animal Cognition and Behavior

Support: The Hartwell Foundation

Weill Cornell Postdoctoral Fellowship

Title: The role of cacna1c in cognitive functioning

Authors: *Z. R. DARUWALLA, D. FISCHER, A. RAJADHYAKSHA;
Pediatric Neurology, Weill Cornell Med. Col., New York, NY

Abstract: Genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) in the *CACNA1C* gene that have been associated with neuropsychiatric disorders including schizophrenia, bipolar disorder, autism spectrum disorder and attention deficit hyperactivity disorder. Common to these neuropsychiatric disorders are impairments in cognitive functioning. Utilizing a forebrain-specific *cacna1c* conditional knockout mouse in which there is a loss of *cacna1c* in the CamK2-expressing glutamatergic cells of the forebrain we have identified deficits in social behavior together with deficits in PFC-specific learning and memory tasks. Indeed, targeted knockdown of *cacna1c* specifically in the adult PFC recapitulated the deficit in social behavior but not in learning and memory suggesting a developmental role of *cacna1c* in learning and memory. Through electrophysiological recordings and biochemical techniques we are currently studying the neurobiology underlying these cognitive deficits in the PFC of forebrain-specific *cacna1c* knockout mice in comparison to the PFC-specific adult *cacna1c* knockdown mice. The idea is to identify the common or diverging pathophysiology in the PFC that may be mediating the social deficits observed in both these mouse models. We are also working towards identifying a pharmacological reversal of the behavioral deficits, specifically the social impairments that we observed in the forebrain-specific *cacna1c* knockout mice and the PFC-specific adult *cacna1c* knockdown mice. Overall, this study has identified a novel role of *cacna1c* in PFC-driven cognitive functioning and will highlight the anatomical and neurobiological mechanisms mediating these behaviors in an effort to provide better therapeutic strategies for treating cognitive impairments in neuropsychiatric patients.

Disclosures: Z.R. Daruwalla: None. **D. Fischer:** None. **A. Rajadhyaksha:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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Weill Cornell Autism Research Program

Title: Altered markers of synaptic development and learning and memory deficits in the cereblon knockout mouse model of intellectual disability

Authors: D. K. FISCHER, C. C. BAVLEY, *A. M. RAJADHYAKSHA;
Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY

Abstract: *Cereblon* (*CRBN*), the human gene on chromosome 3p26.2, has been found to be associated with a non-syndromic, autosomal recessive type of mild mental retardation/intellectual disability (ID). Previous work from our lab using male *Crbn* knockout (*Crbn*$^{-/-}$) mice has shown deficits in hippocampal learning and memory using the Morris water maze, Y-maze, and fear conditioning behavior tests. These behavioral findings using the *Crbn*$^{-/-}$ animal model mirror the human condition, suggesting that this model is an effective tool for investigating the molecular mechanisms associated with hippocampal deficits in intellectual disability.

Hippocampal circuitry in mice is formed during critical periods of development, particularly postnatal days 0-21 (P0-P21). It is during this period that both excitatory (E) and inhibitory (I) synapses are meticulously formed and pruned, thereby shaping the functionality of the hippocampus necessary for learning and memory. Notably, imbalance in E/I synaptic activity has been directly implicated in neurodevelopment disorders with deficits in learning and memory. To begin to examine altered synaptic development during the critical growth period of *Crbn*$^{-/-}$ mice, we examined synaptic protein levels in P7 hippocampal tissue. Examination of excitatory and inhibitory synaptic markers revealed significantly higher levels of VGLUT2 protein levels in P7 *Crbn*$^{-/-}$ mice compared to wildtype littermates with no difference in VGLUT1 or the inhibitory marker, VGAT. Examination of markers of synapse number revealed higher levels of presynaptic marker synaptophysin but not of the postsynaptic marker PSD-95. We also examined postsynaptic synaptic adhesion molecules, neuroligin (NLGN) 1-4, critical for synaptogenesis, in P7 *Crbn*$^{-/-}$ mice hippocampal tissue. Interestingly, only the inhibitory synapse, NLGN-2 levels was altered, with significantly higher levels in *Crbn*$^{-/-}$ mice compared to wildtype littermates. Ongoing studies are investigating molecular mechanisms of altered synaptic markers, as an abundance of synapses may cause abnormalities in neurodevelopment and mediate the learning and memory deficits observed in *Crbn*$^{-/-}$ *mice.*

Disclosures: **D.K. Fischer:** None. **C.C. Bavley:** None. **A.M. Rajadhyaksha:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Title: Loss of MeCP2 in cholinergic neurons causes part of RTT-like phenotypes via the alpha7 receptor in hippocampus

Authors: *Y. ZHANG[1], S.-X. CAO[1], P. SUN[1], H.-Y. HE[1], C.-H. YANG[1], X.-J. CHEN[1], C.-J. SHEN[1], X.-D. WANG[1], Z. CHEN[1], D. K. BERG[2], S. DUAN[1], X.-M. LI[1];
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Abstract: Mutations in the X-linked *MECP2* gene cause Rett syndrome (RTT), an autism spectrum disorder characterized by impaired social interactions, motor abnormalities, cognitive defects and a high risk of epilepsy. Because decreases in cholinergic markers are correlated with increases in clinical severity for patients with RTT, we hypothesized that dysfunction in cholinergic function is involved in RTT. Here, we showed that conditional deletion of Mecp2 in cholinergic neurons caused part of RTT-like phenotypes, which could be rescued by re-expressing Mecp2 in the basal forebrain (BF) cholinergic neurons rather than in the caudate putamen (CPu) of conditional knockout (*Chat-Mecp2*$^{-/y}$) mice. We also found that choline acetyltransferase (ChAT) expression was decreased in the BF and that α7 nicotine acetylcholine receptor (nAChR) signaling was strongly impaired in the hippocampus of *Chat-Mecp2*$^{-/y}$ mice to an extent sufficient to produce neuronal hyperexcitation and increase seizure susceptibility. Application of PNU282987 or nicotine in the hippocampus rescued these phenotypes in *Chat-Mecp2*$^{-/y}$ mice. Taken together, our findings suggest that Mecp2 is critical for normal function of cholinergic neurons and dysfunction of cholinergic neurons can contribute to numerous neuropsychiatric phenotypes.Mutations in the X-linked *MECP2* gene cause Rett syndrome (RTT), an autism spectrum disorder characterized by impaired social interactions, motor abnormalities, cognitive defects and a high risk of epilepsy. Because decreases in cholinergic markers are correlated with increases in clinical severity for patients with RTT, we hypothesized that dysfunction in cholinergic function is involved in RTT. Here, we showed that conditional deletion of Mecp2 in cholinergic neurons caused part of RTT-like phenotypes, which could be rescued by re-expressing Mecp2 in the basal forebrain (BF) cholinergic neurons rather than in

the caudate putamen (CPu) of conditional knockout (*Chat-Mecp2*$^{-/y}$) mice. We also found that choline acetyltransferase (ChAT) expression was decreased in the BF and that α7 nicotine acetylcholine receptor (nAChR) signaling was strongly impaired in the hippocampus of *Chat-Mecp2*$^{-/y}$ mice to an extent sufficient to produce neuronal hyperexcitation and increase seizure susceptibility. Application of PNU282987 or nicotine in the hippocampus rescued these phenotypes in *Chat-Mecp2*$^{-/y}$ mice. Taken together, our findings suggest that Mecp2 is critical for normal function of cholinergic neurons and dysfunction of cholinergic neurons can contribute to numerous neuropsychiatric phenotypes.

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Poster

460. Executive Function: Models of Disorders

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Topic: H.01. Animal Cognition and Behavior

Title: Effect of the sigma-1 receptor selective compound LS-1-137 on the DOI-induced head twitch response in mice

Authors: *R. R. LUEDTKE, Ph.D.[1], M. MALIK[2], C. RANGEL-BARAJAS[3], R. H. MACH[4]; [1]Dept Pharmacol & Neurosci, Univ. North Texas Hlth. Sci. Cter, Fort Worth, TX; [2]Ctr. for Neurosci. Discovery, Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [3]Dept. of Psychological & Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [4]Radiochemistry Laboratory, Dept. of Neurol., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Several receptor mediated pathways have been shown to modulate the murine head twitch response (HTR). However, the role of sigma receptors in the murine (±)-2,5-dimethoxy-4-iodoamphetamine (DOI)-induced HTR has not been previously investigated. We examined the ability of LS-1-137, a novel sigma-1 vs. sigma-2 receptor selective phenylacetamide, to modulate the DOI-induced HTR in DBA/2J mice. We also assessed the *in vivo* efficacy of reference sigma-1 receptor antagonists and agonists PRE-084 and PPCC. The effect of the sigma-2 receptor selective antagonist RHM-1-86 was also examined. Rotarod analysis was performed to monitor motor coordination after LS-1-137 administration. Radioligand binding techniques were used to determine the affinity of LS-1-137 at 5-HT2A and 5-HT2C receptors. LS-1-137 and the sigma-1 receptor antagonists haloperidol and BD 1047 were able to attenuate a DOI-induced HTR, indicating that LS-1-137 was acting *in vivo* as a sigma-1 receptor antagonist.

LS-1-137 did not compromise rotarod performance within a dose range capable of attenuating the effects of DOI. Radioligand binding studies indicate that LS-1-137 exhibits low affinity binding at both 5-HT2A and 5-HT2C receptors. Based upon the results from these and our previous studies, LS-1-137 is a neuroprotective agent that attenuates the murine DOI-induced HTR independent of activity at 5-HT2 receptor subtypes, D2-like dopamine receptors, sigma-2 receptors and NMDA receptors. LS-1-137 appears to act as a sigma-1 receptor antagonist to inhibit the DOI-induced HTR. Therefore, the DOI-induced HTR can be used to assess the *in vivo* efficacy of sigma-1 receptor selective compounds.

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Poster

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Title: Male-specific deficits in natural reward learning in a mouse model of 16p11.2 hemideletion

Authors: *N. M. GRISSOM[1,2], S. MCKEE[1,2], H. SCHOCH[3], N. BOWMAN[1], R. HAVEKES[4], W. O'BRIEN[1], E. MAHRT[5], K. COMMONS[6], C. PORTFORS[5], T. NICKL-JOCKSCHAT[7], T. REYES[2], T. ABEL[1];
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Abstract: Neurodevelopmental disorders, including autism spectrum disorders (ASD), are highly male biased, but the underpinnings of this are unknown. Striatal dysfunction has been strongly implicated in the pathophysiology of neurodevelopmental disorders, raising the question of whether there are sex differences in how the striatum is impacted by genetic risk factors linked to neurodevelopmental disorders. Here, we report male-specific deficits in striatal function

important to reward learning in a mouse model of 16p11.2 hemideletion, a genetic mutation that is strongly associated with risk of neurodevelopmental disorders, particularly autism and ADHD. We find that male, but not female, 16p11.2 deletion animals show impairments in reward-directed learning and maintaining motivation to work for rewards. Male, but not female, deletion animals overexpress mRNA for dopamine receptor 2 and adenosine receptor 2a in the striatum, markers of medium spiny neurons signaling via the indirect pathway, associated with behavioral inhibition. Despite equivalent effects in males and females on the mRNA levels of genes located within the 16p11.2 region in the striatum, including the kinase ERK1, hemideletion males show *increased* activation in the striatum for ERK1 at baseline and in response to sucrose, a signaling change associated with decreased striatal plasticity. In contrast, we find that hemideletion females show increased protein for ERK1 as well as the related kinase ERK2, and no change in phosphorylation either at baseline or in response to sucrose. These data indicate male-specific vulnerability in the mechanisms regulating intracellular signaling in the brain as a result of a genetic lesion.

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Poster

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Title: Cortical GluN2B contribution to reversal learning in Prenatal Alcohol Exposure

Authors: *K. L. MARQUARDT[1], J. CAVANAGH[2], K. CALDWELL[1], J. BRIGMAN[1,3]; [2]Dept. of Psychology, [1]Univ. of New Mexico, Albuquerque, NM; [3]New Mexico Alcohol Res. Ctr., UNM Hlth. Sci. Ctr., Albuquerque, NM

Abstract: With a prevalence rate of 2-5%, Fetal Alcohol Spectrum Disorders (FASD) are a leading cause of preventable developmental disability in the U.S. FASD has a high societal cost, with estimates that as many as 46% of adolescents with FASD end up incarcerated, and 29% end

up being treated for alcohol and drug abuse by the age of 21. These outcomes are the consequence of individuals being unable to alter actions appropriately, leading to repetitive maladaptive behaviors. We have demonstrated that mice given prenatal alcohol exposure (PAE; daily maternal BAC ~85 mg/dL) have impaired behavioral flexibility, but not learning, demonstrated by an increase in perseverate responses on a touch-screen discrimination-reversal paradigm. Interestingly, conditional cortico-hippocampal knockout of the GluN2B (GluN2B^{CxNULL}) subunit of the N-methyl-D-aspartate (NMDA) receptor also results in selective disruption of reversal, but not learning. It is well known that ethanol can directly inhibit NMDA receptors and that GluN2B is highly expressed during gestation, where it may be modulated by PAE. Optimal behavioral flexibility requires both the dorsal striatum (dS) for formation of stimuli-reward associations, and the orbital frontal cortex (OFC) for reversal. We show that PAE reduces the expression of GluN2B in the OFC, but not in other subdomains of the frontal cortex, suggesting a unique role for GluN2B subunits in reversal learning. To further characterize the role of GluN2B subunits in behavioral flexibility we utilized dual region *in vivo* electrophysiology to record in both the OFC and dS during learning and reversal stages of a discrimination-reversal touch-screen paradigm in both PAE and GluN2B^{CxNULL} mice and controls. Analysis of local field potential oscillations revealed differences in phase coherence in both OFC and dS for PAE animals, but only in dS for GluN2B^{CxNULL}. Suggesting changes in modulation of the dS by the OFC during reversal in both models that is GluN2B subunit dependent. Inter site phase synchrony, a measure of functional connectivity, showed decreased connectivity in PAE animals, but delayed connectivity in GluN2B^{CxNULL}. Our data suggest that PAE deficits may be at least partially due to loss of GluN2B levels in the cortex, but it is selective to loss in the OFC which drives the deficit. By understanding the cause of deficits in PAE, and by utilizing translatable touch screen tasks, we will be able to develop better treatment tools for FASD.

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Poster

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SFB1134

Title: Alcohol induced mGluR2 deficit in the prefrontal cortex leads to behavioral inflexibility in rats

Authors: *S. PFARR[1], M. L. KLEE[1], M. W. MEINHARDT[1], N. MEIER[3], O. VON BOHLEN UND HALBACH[3], M. SCHNEIDER[1], R. L. BELL[4], K. SCHÖNIG[2], W. H. SOMMER[1]; [1]Psychopharmacology, [2]Molecula Biol., Central Inst. of Mental Hlth., Mannheim, Germany; [3]Inst. für Anatomie und Zellbiologie, Universitätsmedizin, Greifswald, Germany; [4]Inst. of Psychiatric Res., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The medial prefrontal cortex (mPFC) is critically involved in cognitive flexibility and top down control over behavior. Both, in humans and animals, excessive alcohol use causes damage in the prefrontal cortex. In a previous study we found that alcohol dependent rats lost control over their alcohol seeking behavior, which was mediated by a metabotropic glutamate receptor 2 (mGluR2) deficit in the infralimbic (IL) – Nucleus Accumbens (NAc) Shell projection [1]. An open question is whether this molecular mGluR2 deficit has also functional consequences on executive functions.

Here, we used chronic intermittent alcohol vapor exposure (CIE) to induce alcohol dependence in adult Wistar rats. Investigations were done after a period of prolonged abstinence (3 weeks), where animals show persistent neuronal and behavioral adaptations here referred to as 'post-dependent' state (PD) [2]. First we demonstrate a distinct profile of structural changes in the mPFC – NAc circuit in PD rats characterized by significantly increased spine density in mPFC sub regions, but decreased spine density in the NAc. Further, in an Attentional Set Shifting Task (ASST) PD rats showed significant impairments compared to control animals, when the rules of the test were changed. This inability to change their strategy indicates a reduced cognitive flexibility of the PD rats. The deficit in ASST performance was partially reversed after re-establishing mGluR2 expression in the IL of PD rats by viral vector mediated gene transfer, while overexpression of mGluR2 in control rats had no behavioral consequence. On the other hand no deficit in ASST performance was found in a functional mGluR2 knockout line, the Indiana Alcohol preferring rats (Indiana P rats), compared to their respective control strain (Indiana NP rats). This indicates a possible functional compensation of the genetic mGluR2 deficit. Furthermore, first experiments using a viral mGluR2 knockdown approach in the IL did not produce a deficit on ASST in Wistar rats.

These data further establish the long lasting impact of a history of alcohol dependence on mPFC function, especially on cognitive flexibility. mGluR2 deficit in the IL emerges as a molecular mechanism mediating alcohol induced cognitive impairments. However, mGluR2 deficit alone does not seem to be sufficient to induce this pathology, but likely requires interaction with other, so far unknown alcohol induced molecular alterations.

[1] Meinhardt, MW. J Neurosci 2013, 33(7):2794-806 [2] Meinhardt, MW. Addict Biol 2015, 20(1):1-21.

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Poster

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Title: Modeling exposure therapy in rats: fear extinction-induced infralimbic protein synthesis underlies reversal of chronic stress-induced cognitive inflexibility

Authors: *E. A. FUCICH[1], D. PAREDES[2], D. A. MORILAK[2];
[1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [2]Dept. of Pharmacology, Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Stress-related psychiatric disorders, like depression or post-traumatic stress disorder, are prevalent yet poorly treated. These disorders share cognitive flexibility deficits associated with medial prefrontal cortex (mPFC) dysfunction. Psychotherapies invoking cognitive flexibility can be efficacious even in pharmacotherapy-resistant patients, although, as with pharmacotherapies, response to psychotherapy can be incomplete, some patients do not respond, and relapse remains an issue. Thus, understanding the neurobiological mechanisms underlying its efficacy could inform more rapid, efficacious, or long-lasting behavioral therapies, or could inform the development of adjunct treatment strategies designed to improve its effect. Pre-clinically, we have shown that chronic unpredictable stress (CUS) causes deficits in mPFC-mediated cognitive flexibility on the attentional set-shifting test (AST). We have shown that fear extinction learning, which engages mPFC cognitive flexibility and conceptually resembles exposure therapy for PTSD in humans, can model exposure therapy in rats by improving set-shifting performance in the AST that has been compromised by chronic stress (SfN Abstract 468.07, 2014). This study tested whether extinction-induced mPFC protein translation was necessary for the reversal of stress-compromised set-shifting by extinction therapy. After 2 weeks of CUS or control treatment, rats received microinjections of the protein synthesis inhibitor anisomycin or saline vehicle in the ventral mPFC (i.e., the infralimbic region, IL) followed by fear extinction training or control treatment. They were tested on AST 24h later. Anisomycin delivered to IL cortex blocked the rescue of mPFC-mediated cognitive flexibility by extinction in stressed rats. By contrast, control injections of anisomycin into the PrL subregion of

mPFC dorsal to the IL did not attenuate the beneficial effects of extinction. Ongoing studies are examining changes in plasticity-related proteins in IL tissue of rats following fear extinction. These results suggest that fear extinction-induced protein translation underlies the therapeutic effect of fear extinction. Such processes may be important to the beneficial effects on cognition that have been compromised by chronic stress, and may suggest targets for the development of adjunct pharmacological tools to enhance the efficacy of behavioral therapy.

Disclosures: E.A. Fucich: None. **D. Paredes:** None. **D.A. Morilak:** 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; H Lundbeck A/S. F. Consulting Fees (e.g., advisory boards); Lundbeck Research, USA.

Poster

460. Executive Function: Models of Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 460.09/KKK66

Topic: H.01. Animal Cognition and Behavior

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Title: Modeling exposure therapy in rats: fear extinction-induced infralimbic activity underlies reversal of chronic stress-induced shift towards passive coping

Authors: *D. A. MORILAK, E. FUCICH, M. SAUNDERS;
Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Stress-related psychiatric disorders, like depression or post-traumatic stress disorder, are highly prevalent yet poorly treated. These disorders share many dimensions, including maladaptive avoidant coping strategies, thought to be modulated by medial prefrontal cortex (mPFC) activity. Psychotherapies invoking mPFC activity can be efficacious even in pharmacotherapy-resistant patients, although, as with pharmacotherapies, patient response and relapse remain issues. Identifying the neurobiological mechanisms underlying psychotherapy's

efficacy could lead to more rapid, efficacious, or long-lasting treatments. Pre-clinically, chronic unpredictable stress (CUS) induces a shift towards passive coping behavior in rats, similar to avoidant coping behaviors seen in patients with stress-related psychiatric illness. We have previously shown that fear extinction, which engages mPFC and closely resembles exposure therapy for PTSD, can model psychotherapy in rats by restoring active coping in the shock probe defensive burying (SPDB) test after chronic stress (SfN Abstract 468.07, 2014). In this study, we tested the hypothesis that mPFC activity during extinction is necessary for its beneficial effect in reversing CUS-compromised coping behavior in the SPDB test. To test the necessity of mPFC glutamatergic cell activity during extinction for its therapeutic impact, rats received AAV microinjections into the ventral mPFC (infralimbic cortex, IL) to express the Gi-coupled designer receptor exclusively activated by designer drug (DREADD) hM4Di or control GFP protein, driven by a CaMKII promoter. After four weeks of viral expression, including 2 weeks of CUS or control treatment, rats received an IP injection of the designer drug clozapine-n-oxide (CNO, 1mg/kg) followed by extinction or control treatment 30 min later. They were tested on SPDB 24h later. Immunohistochemical analyses of IL cortex showed that >60% of extinction-induced cFos was colocalized with CaMKII. In rats expressing the inhibitory hM4Di receptors in CaMKII+ neurons in IL, CNO reduced extinction-induced cFos expression by >60%. CNO also blocked the rescue of active coping by extinction in the SPDB test in stressed animals expressing hM4Di. These results suggest that mPFC activity underlies the effect of extinction on mPFC-modulated coping behavior. This study further shows that fear extinction is a useful model of exposure therapy, allowing us to investigate neural mechanisms responsible for its therapeutic effects.

Disclosures: **D.A. Morilak:** 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; H Lundbeck A/S. F. Consulting Fees (e.g., advisory boards); Lundbeck Research, USA. **E. Fucich:** None. **M. Saunders:** None.

Poster

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DARPA SIMPLEX N66001-15-C-4032

ARO W911NF-12-1-0594

Title: Alter neuronal ensembles in a neuronal overproduction mouse model of autism

Authors: *W. FANG, R. YUSTE;
Columbia Univ., New York, NY

Abstract: The variability in brain connectivity likely underlies individual differences in brain function. However, the causal link between variability in brain development and function remains poorly understood. In particular, it is not known how the brain regulates neuronal production and/or compensates if fewer or more neurons are generated. This is not an academic question since there are significant differences in brain size and neuron numbers across individuals. In addition, pathologies such as autism or mental retardation are associated with abnormal neuron numbers. We investigated a new mouse model of autism with overproduction of neurons in the neocortex and explored how surplus of neurons leads to differences in functional connectivity. We focused on neuronal ensembles, which are composed of specific groups of functionally related neurons and could represent building blocks of cortical function. To map neuronal ensembles and identify their spatiotemporal layouts in vivo, we performed two-photon calcium imaging of populations of primary visual cortical neurons. We find more and larger neuronal ensembles in the neocortex of autism mouse model brains. In addition, coactive neurons in these ensembles are more localized. These findings suggest that increased number of neurons generates more local functional modules, and agree with clinical studies showing stronger functional connectivity in autistic patients. Our work thus reveals that, in a mouse model of autism, neuronal overproduction leads to altered functional connectivity in the neocortex, providing developmental insights into the pathophysiology of autism.

Disclosures: **W. Fang:** None. **R. Yuste:** None.

Poster

460. Executive Function: Models of Disorders

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Topic: H.01. Animal Cognition and Behavior

Support: TMARC

Title: Methamphetamine rescues latency of reward collection in TAT transgenic mice while not affecting probabilistic learning, motivation, or exploratory behavior compared to controls.

Authors: *M. B. MILIENNE-PETIOT[1,3], D. S. DEBEN[3], J. W. YOUNG[1], A. MINASSIAN[1], T. TMARC[2];
[1]Psychiatry, [2]UCSD, La Jolla, CA; [3]Pharmacol., Utrecht Univ., Utrecht, Netherlands

Abstract: Introduction - Human Immunodeficiency Virus (HIV) is a worldwide problem with a prevalence of 0.8%. Due to the chronic nature of the disease, it can lead to HIV-associated neurocognitive disorders (HAND). HIV sufferers often abuse methamphetamine (METH), with claims of alleviation of symptoms by users. One important protein of HIV and HAND is the Transactivator of Transcription (TAT) as it can be expressed in the brain. Importantly, both TAT and METH inhibit dopamine transporter (DAT) function, and so may exert synergistic effects on behaviors relevant to HIV risk transmission such as reward-seeking and motivation. Confirmation of synergistic effects require testing in experimental animals, and can be conducted in inducible TAT mice. Methods - 59 male transgenic mice (30 wildtype (WT); 29 TAT+) were tested in the Probabilistic Learning (PL) task, Progressive Ratio Breakpoint (PRB) task, and in the Behavioral Pattern Monitor (BPM). Mice were treated with an increasing dose METH regimen (or vehicle) for 4 weeks before receiving 7 days of doxycycline injections to induce TAT expression. Results - Neither genotype, METH, or their interactions affected BPM measures, effortful motivation, or PLT accuracy. A main effect of METH on Mean Reward Latency (MRL) in the PRB was observed [$F_{(1,46)}=4.9$, $p<0.05$], but no interaction with genotype ($F<1$, ns). In the PL task, there was an interaction between genotype and METH for MRL [$F_{(1,49)}=4.6$, $p<0.05$]. Vehicle-treated TAT expressing mice exhibited significantly higher MRL compared to WT mice ($p<0.01$), an effect not observed after METH treatment (ns). Conclusion - TAT protein expression in mice slowed the collection of rewards, without affecting choice latency. Pretreatment with METH however, rescued the latency to collect rewards. These data may relate to HIV sufferers seeking METH to alleviate reward apathy. No other interactive effects were observed however, in probabilistic learning, effortful motivation, or exploratory behavior. Future studies should further evaluate the effects of concomitant HIV and drug use on other types of behavior.

Disclosures: M.B. Milienne-Petiot: A. Employment/Salary (full or part-time): UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; TMARC. **D.S. Deben:** None. **J.W. Young:** A. Employment/Salary (full or part-time): UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; TMARC. **A. Minassian:** A. Employment/Salary (full or part-time): UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; TMARC. **T. Tmarc:** None.

Poster

460. Executive Function: Models of Disorders

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Title: Pavlovian and dopaminergic influences on the development and escalation of 'compulsive' checking behaviour in rats: implications for models of obsessive compulsive disorder (OCD).

Authors: *D. M. EAGLE[1], C. SCHEPISI[1], S. CHUGH[1], S. DESAI[1], S. HAN[1], T. HUANG[1], J. LEE[2], C. SOBALA[1], W. YE[1], T. W. ROBBINS[1];
[1]Univ. Cambridge, Cambridge, United Kingdom; [2]Univ. Bristol, Bristol, United Kingdom

Abstract: Compulsive checking is a common, debilitating symptom of OCD. For healthy people, checking is a functional, appropriate behaviour, and it is not clear how this behaviour develops into the excessive/compulsive checking that is found in OCD. We propose that prior Pavlovian-conditioned links between checking and positive outcomes might influence the subsequent development of high levels of checking behaviour.
In previous studies, quinpirole (dopamine D2/3R agonist) increased checking responses of rats (in behavioural tests including the Observing Response Task (ORT)). In the ORT, rats use a lever (checking response) to access information about which of two other levers gives food reward. In the current study, prior to ORT training, 24 rats received Pavlovian Autoshaping training (Pav), to develop an association between the checking response lever and 'reward' pellets (24 rats received Control (Con) training). When treated with quinpirole (QNP; 0.5 mg/kg, 10 sessions) on the ORT, Pav rats significantly increased checking, whereas Con rats' checking was not increased by QNP. The interaction between Pav and QNP increased checking both during the QNP treatment phase and during 10 non-treatment days post-QNP, showing potential long-term effects of this treatment interaction. Functional checking (responses to turn the light on) was affected to a greater extent than dysfunctional checking (checking responses while the light was illuminated and having no further consequence).
OCD checking may also be influenced by uncertainty. When uncertainty was increased in the

ORT, previously Pav-trained rats selectively escalated dysfunctional checking compared with Con rats. Further investigation showed that rats defined by published criteria as 'sign-tracking' escalated dysfunctional checking to a far greater extent than 'goal-tracking' rats (which made no more dysfunctional checking responses than Con rats).
These results support our hypothesis that Pavlovian conditioned links between checking and positive outcomes affect:
i) dopaminergic mechanisms controlling checking;
ii) uncertainty-induced escalation of dysfunctional checking, especially for 'sign-trackers'.
These findings give us insight into how checking might transform from functional behaviour to excessive and dysfunctional behaviour in OCD.

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Poster

460. Executive Function: Models of Disorders

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Support: Wellcome Trust Programme Grant 089589/Z/09/Z

Title: Cortico-striatal regions of the rat and checking-like behaviour: Dissociable effects of lesions to the orbitofrontal cortex, medial prefrontal cortex, nucleus accumbens core and dorsal striatum in an operant observing response task

Authors: *L.-S. D'ANGELO[1,2], D. M. EAGLE[1,2], C. M. COMAN[3], T. W. ROBBINS[1,2];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Behavioural and Clin. Neurosci. Inst., Cambridge, United Kingdom; [3]Univ. Paris-Saclay, Paris, France

Abstract: Excessive checking is a common, debilitating symptom of obsessive-compulsive disorder (OCD). To further examine cognitive processes underpinning checking behaviour, and clarify how and why checking develops, we designed a novel operant paradigm for rats, the observing response task (ORT) (Eagle et al., 2014). In the present study, the ORT was used was used to investigate the functional role that brain regions implicated in a neuroanatomical circuit of OCD may play in compulsive checking behaviour. There is evidence that OCD is related to dysfunction of cortical-basal ganglia systems (Graybiel & Rauch 2000; Menzies et al. 2008). The experimental approach involved excitotoxic lesions of the orbital frontal cortex (OFC), medial prefrontal cortex (mPFC), nucleus accumbens core (NAcbC) and dorsal striatum (DStr), brain

regions considered to be of relevance to OCD. In the ORT, rats pressed an 'observing' lever for information about the location of an 'active' lever that provided food reinforcement. OFC lesions had no effect on observing but did impair instrumental choice behaviour in a pattern of deficits suggesting that OFC lesions disrupted the ability to use information from observing appropriately. mPFC lesions selectively increased functional observing without affecting other task measures. Rats with NAcbC lesions made significantly more observing responses than control shams, both for functional observing lever presses and non-functional observing lever presses. DStr lesions had no effect on observing, but reduced both active and inactive lever presses. These results provide novel information about the role of cortico-striatal regions in checking-related behaviour and accordingly provide insight into possible pathophysiology that underlies the development of compulsive checking observed in OCD.

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Poster

460. Executive Function: Models of Disorders

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Title: Effects of cola nitida acetone extract on brain sodium-potassium adenosine triphosphatase activity, spatial memory in healthy and streptozotocine induced diabetic female wistar rats

Authors: *A. O. IMAMFULANI, K. O. SANUSI, B. V. OWOYELE;
Physiol., Univ. of Ilorin, Ilorin, Nigeria

Abstract: Background: Individuals with type 1 diabetes have been reported to show modest deficit on wide range of neuropsychological tests compared with nondiabetic subjects. This study was carried out to investigate the effects of acetone extract of Cola nitida on brain Na+/K+ ATPase activity and spatial memory of STZ induced diabetic Wistar rats.
Methods: Female Wistar rats were used for this study and were randomly distributed into six (6) groups with seven animals in each group. Animals in group 1 were control and administered normal saline; Group 2 animals were diabetic group and Group 3 were diabetic animals and administered 50mg/kg of kola nut extract per day; Group 4 were diabetic animals and administered 100mg/kg of kola nut extract per day; Group 5 were healthy animals and administered 50mg/kg of kola nut extract per day; Group 6 were healthy animals and administered 100mg/kg of kola nut extract per day. After three (3) weeks of administration, the spatial memory and Na+/K+ ATPase activity were analysed.

Results: The result of the experiment shows a significant increase in Na+/K+ ATPase activity of groups 3 and 4 administered 50mg/kg and 100mg/kg of kola nut extract respectively when compared with group 2
($p<0.05$), and a significant increase ($p<0.05$) in Na+/K+ ATPase activity of groups 5 and 6 administered 50mg/kg and 100mg/kg of kola nut extract respectively when compared with group 1 ($p<0.05$).
Conclusion: this study shows that kola nut extract has positive effect on brain Na+/K+ ATPase activities and spatial memory of STZ-induced diabetic Wistar rats.

Disclosures: **A.O. Imamfulani:** None. **K.O. Sanusi:** None. **B.V. Owoyele:** None.

Poster

460. Executive Function: Models of Disorders

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Topic: H.01. Animal Cognition and Behavior

Support: RF SUNY BDAA grant

Title: Behavioral and physiological effects of excitatory DREADD expression in the rat medial prefrontal cortex: Lack of evidence for neuronal activation with clozapine-N-oxide

Authors: *K. ISHIWARI[1], A. M. GEORGE[1], C. D. MARTIN[1,2], K. A. HAUSKNECHT[1], R.-Y. SHEN[1], S. HAJ-DAHMANE[1], J. B. RICHARDS[1];
[1]Res. Inst. on Addictions, [2]Psychology, Univ. at Buffalo, Buffalo, NY

Abstract: Habituation of reinforcer effectiveness (HRE) may be a fundamental property of reinforcing stimuli. Abnormal HRE due to genetic and/or environmental factors may play a role in dysfunctional behavioral regulation seen in such disease states as ADHD, autism, obesity, and drug abuse. We have previously shown that operant responding for a visual sensory stimulus (light onset) rapidly habituates in rats[1]. Electrophysiological evidence indicates that novel sensory stimuli increase burst firing of midbrain dopaminergic (DA) neurons, which rapidly decreases with repeated presentation of the stimulus[2]. The medial prefrontal cortex (mPFC) modulates the activity of midbrain DA neurons through multiple pathways, exerting both excitatory and inhibitory influences. To elucidate the role of the mPFC in HRE, the present study attempted to examine effects of chemogenetic neuronal activation of the mPFC using a designer receptor exclusively activated by designer drugs (DREADD) on HRE of a light stimulus. Three groups of male rats were tested for snout poke responding for light onset in six 18-min daily sessions. One group of rats (n = 12) expressed the excitatory DREADD (AAV5-CaMKIIα-

hM3Dq-mCherry) in the mPFC and were injected with clozapine-N-oxide (CNO; 3 mg/kg, i.p.) 30 min before each of the six light reinforcement sessions. A second group (n = 10) also expressed hM3Dq in the mPFC but received saline vehicle. A third group (n = 7) had sham surgery in the mPFC and received CNO. The hM3Dq/CNO rats displayed larger degrees of both between- and within-session habituation in responding for the light relative to the hM3Dq/saline rats. However, the sham-operated rats receiving CNO also habituated to the same degree as the hM3Dq/CNO rats. Moreover, whole-cell patch-clamp recordings in brain slices showed that bath application of CNO (10-30 μM) did not significantly increase the excitability of layer V mPFC pyramidal neurons expressing hM3Dq/mCherry (n = 6 cells). Taken together, our results suggest that CNO, or its metabolites, had attenuating effects on responding for the light that were not DREADD-mediated.

1. Lloyd, D.R., Medina, D.J., Hawk, L.W., Fosco, W.D., and Richards, J.B. (2014). Habituation of Reinforcer Effectiveness. Front Integr Neurosci. 7:107. doi: 10.3389/fnint.2013.00107.
2. Redgrave, P. and Gurney, K. (2006). The short-latency dopamine signal: A role in discovering novel actions? Nat. Rev. Neurosci. 7: 967-975. doi: 10.1038/nrn2022.

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Poster

460. Executive Function: Models of Disorders

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Topic: H.01. Animal Cognition and Behavior

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Title: Timing and temporal discounting in a model of aging-related parkinsonism

Authors: ***M. BUHUSI**[1], A. MANJUNATH[2], A. R. MATTHEWS[1], K. OLSEN[1], J. CARLSON[1], J. YANG[3], C. V. BUHUSI[1];
[1]Dept. Psychology, [2]Dept. Engin., Utah State Univ., Logan, UT; [3]Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Parkinson's Disease, the most common neurodegenerative movement disorder, is also associated with deficits in timing, planning, working memory and inhibitory control, suggestive of frontal executive dysfunction due to alterations in dopaminergic circuits. Glial-derived neurotrophic factor (GDNF) is essential for regulating DA release in the basal ganglia and the survival of DA neurons, and GDNF-deficient mice are considered an animal model for aging-

related Parkinsonism. Therefore, we assessed interval timing in the peak-interval (PI) procedure and decision making in the temporal discounting (TD) task as measures of executive function in GDNF-heterozygous (HET) mice, having a partial reduction of GDNF levels, and their wild-type littermate controls (WT). As expected, relative to WT controls, a significant impairment in timing was found in aged, but not in adult presymptomatic GDNF-HET mice. Also, adult presymptomatic GDNF-HET showed no deficits in TD relative to their WT controls. However, following chronic unpredictable stress, adult GDNF-HET mice showed increased impulsive choice indexed by a reduction in percent larger-later choices and a reduction in area under the TD curve. Moreover, adult stressed GDNF-HET mice, but not their WT controls, showed decreased neuronal activation (number of cFos positive neurons) in the orbitofrontal cortex and nucleus accumbens, suggestive of maladaptive response to stress, in accord with recent reports linking GDNF to resilience to stress. Interestingly, impulsivity (indexed by area under the TD curve) negatively correlated with activation of the nucleus accumbens core and shell, but not with orbitofrontal activity. Taken together, these results support interval timing and temporal discounting as markers of cognitive impairment in animal models of Parkinsonism, and identify GDNF-deficient mice as a double-hit (gene x environment) model of stress-related cognitive impairment.

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Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

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Title: Increased hippocampal neurogenesis is associated with worse contextual memory.

Authors: *J. JORDAN[1], S. SCHWARTZ[3], I. VORONINA[4], K. LIN[5], C. HARDING[5,2,1], C. PYTTE[1,3,2], A. WINTER[6];
[1]Biol., [2]Psychology, Grad. Center, The City Univ. of New York, New York, NY; [3]Psychology, Queens College, The City Univ. of New York, Flushing, NY; [4]Chem., [5]Psychology, Hunter College, The City Univ. of New York, New York, NY; [6]Paul D Schreiber High Sch., Port Washington, NY

Abstract: The hippocampus is essential for the formation of new spatial and contextual memories and is a site of adult neurogenesis. Exactly how newborn neurons contribute to hippocampal-dependent memory has been widely debated. Evidence has shown that ablating neurogenesis impairs contextual memory, a hippocampal-dependent form of memory, and it is often assumed that increased neurogenesis improves hippocampal memory. To investigate the relationship between new neurons and strength of contextual memories, we injected mice with the mitotic marker BrdU (2 times per day for 4 days). Two weeks later, mice were fear-conditioned to a particular context, then re-exposed to the fearful context both at a 30-minute and at a 24-hour delay. We subtracted movement during the recall sessions from baseline movement measured before shock was delivered during the training session; a larger difference between these two values indicates better contextual memory. We sacrificed mice 4.5 weeks after the last BrdU injection and used IHC to label BrdU and NeuN, a marker of mature neurons, throughout the dorsomedial dentate gyrus. In separate sections, we labeled doublecortin (DCX), a marker of immature neurons. Surprisingly, we found that numbers of BrdU+ neurons were negatively correlated with contextual memory, both 30 minutes and 24 hours after training. The more new neurons, the worse the mouse's memory. These findings were the same when analyzing new neuron densities across the entire dentate gyrus, as well as specifically in the subgranular zone, the site within the dentate gyrus where new neurons are born. We found no significant correlations between DCX+ neuron density and recall at either time point. No significant correlations were found between DCX+ or BrdU+/NeuN+ neurons and baseline movement before fear conditioning or with auditory fear memory. Although other work has shown that new neurons are needed for new contextual memories, too many may interfere with hippocampal processing. Alternatively, mice that were able to form better memories may have fewer new neurons surviving to maturity. Though we cannot determine causality, a higher density of mature new neurons was associated with worse contextual memory.

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Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

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Program#/Poster#: 461.02/LLL5

Topic: H.01. Animal Cognition and Behavior

Support: R01AG033570

Title: Depletion of adult neurogenesis compromises hippocampal feedback inhibition.

Authors: *C. L. HOLLANDS[1], S. KERNIE[2], O. LAZAROV[1];
[1]The Univ. of Illinois at Chicago, Chicago, IL; [2]Columbia Univ., New York, NY

Abstract: Hippocampal adult neurogenesis is thought to play an essential role in hippocampal plasticity, but the mechanism is not fully elucidated. Here we show that ablation of neurogenesis in the adult brain compromises recognition memory. Ablation of neurogenesis was achieved by treating nestin-δ-HSV-TK mice with valganciclovir. To assess the impact of loss of neurogenesis on the activity of the hippocampal circuitry we examined the expression of the immediate early gene *Egr-1* (*zif268*), as an indicator of neuronal activation following learning. For this purpose, nestin-δ-HSV-TK mice were trained in the pattern separation task, probed for long-term memory and immediately examined for *Egr-1* expression in the hippocampus. We show a significant increase in the number of *Egr-1* expressing neurons in both the CA3 and CA1 regions in nestin-δ-HSV-TK mice with ablated neurogenesis compared to nestin-δ-HSV-TK mice with intact neurogenesis. These results suggest that neurogenesis plays an important role in regulation of feedback inhibition, and that interference with inhibitory hippocampal circuitry following depleted neurogenesis leads to neuronal over activation. This data suggests that deficits in adult neurogenesis may induce deficits in learning and memory.

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Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

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Topic: H.01. Animal Cognition and Behavior

Support: Radford University Department of Psychology

Title: Analysis of gender and strain differences in basal hippocampal neurogenesis in adult rats

Authors: A. M. FORMICA, F. E. GRIFFEY, A. R. DIXON, *D. M. HAYES;
Psychology, Radford Univ., Radford, VA

Abstract: A comprehensive body of research now indicates that neurogenesis occurs throughout adulthood in a variety of species. This process, by which new neurons are born and ultimately become integrated into existing brain circuitry takes place in four stages: cell proliferation, differentiation, maturation, and survival. One of the two primary regions where neurogenesis occurs is the hippocampus, which plays an extensive role in learning and memory. Though investigators have analyzed how various experimental interventions might affect neurogenesis in this brain region, studies have been primarily conducted in male Sprague-Dawley rats despite known differences between the genders as well as rat strain. For instance, androgens have been shown to enhance cell proliferation while estrogens have been shown to ultimately suppress proliferation. Additionally, female rats have been shown to be more susceptible to stress when compared to male rats with stress having been found to inhibit neurogenesis. Furthermore, Long-Evans rats have generally outperformed Sprague-Dawley rats on several hippocampal-dependent learning tasks, such as the Morris Water Maze, where better performance is generally thought to correlate with higher levels of neurogenesis. The current study was therefore designed to investigate baseline levels of neurogenesis using rats of both genders from two separate strains. To that end, adult male and female Sprague-Dawley and Long-Evans rats were perfused without exposure to experimental manipulations. Brains were collected, sliced, and stained for Ki67 immunoreactivity, an endogenous indicator of cell proliferation. Though it was predicted that Long-Evans rats and male rats would show significantly higher levels of neurogenesis than their respective counterparts, preliminary analyses failed to reveal an effect of either strain or gender on the number of Ki67-positive cells found in the hippocampus. Furthermore, there was not a significant interaction between gender and strain on Ki67-positive cell numbers. However, the data does show a trend toward Long-Evans animals displaying increased cell proliferation compared to Sprague-Dawley animals, which may become statistically significant with increased statistical power. Results from this study will extend findings that suggest a need for researchers to take into account various strains and both genders in future studies in order to increase the generalizability of research intended to model human conditions.

Disclosures: **A.M. Formica:** None. **F.E. Griffey:** None. **A.R. Dixon:** None. **D.M. Hayes:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 461.04/LLL7

Topic: H.01. Animal Cognition and Behavior

Support: SFI Grant 12/IA/1537

Title: Lentiviral overexpression of interleukin-1β in the hippocampus induces neurogenesis-associated cognitive deficits in touchscreen learning paradigms

Authors: *C. M. HUESTON, J. F. CRYAN, Y. M. NOLAN;
Univ. Col. Cork, Cork, Ireland

Abstract: Adult neurogenesis within the subgranular zone of the dentate gyrus of the hippocampus is integral for normal cognitive function, and is especially important for spatial memory tasks and pattern separation tasks. Previous studies have demonstrated that acutely elevated levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) in the hippocampus has detrimental effects on some aspects of memory and cognitive function, as well as a negative impact on the proliferation and survival of newly born neurons. Touchscreen-based platforms for testing cognitive function have been developed that allow several different types of memory and learning to be assessed, including pattern separation which can be tested using the location discrimination paradigm. The current study aimed to assess whether long-term increased expression of IL-1β through lentiviral-mediated overexpression of the protein would alter performance in pattern separation using the touchscreen location discrimination test. To accomplish this, adult male Sprague-Dawley rats were first trained to use the touchscreen testing apparatus, and to perform intermediate-separation location discrimination. Once all animals had passed criteria, a lentivirus overexpressing IL-1β and the fluorophore mCherry, or a control virus expressing only mCherry was bilaterally injected into the dorsal dentate gyri of rats, and they were allowed to recover. Two weeks after injection, rats were re-tested on the intermediate location discrimination task to check post-surgical performance. Rats were then introduced to the large and small separation aspects of the pattern separation task. IL-1β overexpression in the dorsal hippocampus resulted in impaired performance in the large, but not the small location discrimination task. Additionally, IL-1β overexpression resulted in a reduction in the number of proliferating new cells within the dentate gyrus as measured by Ki67 expression. The survival and numbers of newly born neurons will also be measured by immunohistochemical analysis using antibodies against DCX and BrdU/NeuN. Together, the results from the current experiment suggest that chronic overexpression of IL-1β in the hippocampus induces a deficit in pattern separation, a hippocampal neurogenesis-dependent cognitive behaviour.

Disclosures: **C.M. Hueston:** None. **J.F. Cryan:** None. **Y.M. Nolan:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 461.05/LLL8

Topic: H.01. Animal Cognition and Behavior

Support: NIH R21-NS085502-02

Title: Exercise-induced changes in NGF are critical for the rescue of spatial working memory and septohippocampal functioning

Authors: *J. M. HALL[1], F. GOMEZ-PINILLA[2], L. Z. YING[2], L. M. SAVAGE[1]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]UCLA, Los Angeles, CA

Abstract: Exercise has shown robust capabilities at improving and rescuing cognitive function in both humans and rodents, which has been assumed to directly involve increases in hippocampal neurotrophins and hippocampal neurogenesis. Our laboratory has recently shown that exercise with an adaption period leads to: 1) a rescue of spatial working memory, 2) a restoration of septohippocampal cholinergic functioning and 3) a selective re-emergence of the Nestin+ cholinergic phenotype (Hall & Savage, 2016). Thus, our aim was to investigate which neurotrophin, brain-derived neurotrophic factor (BDNF), or nerve growth factor (NGF), was responsible for this cholinergic-based recovery. To do so, PTD and control rats were infused with fluorescent latex microspheres into the dorsal hippocampus that were coated with either TrkA-IgG or TrkB-IgG, to sequester free NGF and BDNF (respectively) during exercise. Rats also received ventral hippocampal cannulations for assessment of acetylcholine release during spontaneous alternation. Results indicated that sequestering NGF, but not BDNF, abolished the exercise-induced recovery of spatial working memory and acetylcholine release. Thus, exercise-induced changes in NGF within the septohippocampal pathway represent another avenue for recovery of function for disorders of memory and cognition.

Disclosures: J.M. Hall: None. **F. Gomez-Pinilla:** None. **L.Z. Ying:** None. **L.M. Savage:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 461.06/LLL9

Topic: H.01. Animal Cognition and Behavior

Title: Seizures originating in different brain regions have differential effects on fear memory and the functional integration of seizure-generated granule neurons

Authors: *N. NOGOVITSYN[1], J. J. BOTTERILL[2], H. J. CARUNCHO[3], L. E. KALYNCHUK[4];
[1]Col. of Med., [2]Col. of Psychology, [3]Col. of Pharm. and Nutr., [4]Dept. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Temporal lobe epilepsy is associated with impaired cognition, but the mechanisms by which this occurs remain unclear. Here, we investigated whether seizures that originate in different brain regions have differential effects on hippocampal-dependent learning and memory and the recruitment of adult-generated neurons into memory networks. To accomplish this, we used the kindling model of epilepsy, in which daily brief electrical stimulation of a discrete brain region instigates the gradual development and intensification of motor seizures. We subjected rats to 99 kindling stimulations of limbic (basolateral amygdala and dorsal hippocampus) or non-limbic (caudate nucleus) brain regions. To evaluate the effects of seizures on hippocampal neurogenesis and the integration of adult-generated neurons into memory networks, we performed BrdU pulse-chasing experiments (3 i.p. injections; 24 hours apart) 4-weeks prior to the cessation of kindling. Upon the completion of kindling, rats were subjected to a 4-day hippocampal dependent trace fear conditioning paradigm comprising habituation, training, and memory testing (tone and context, respectively). Approximately 1 hr after the final contextual fear memory retrieval test (day 4), all rats were sacrificed and their brains were prepared for immunohistochemistry. We found that kindling had no effect on the acquisition of hippocampal-dependent fear conditioning; however, limbic-kindled, but not non-limbic kindled, rats showed impaired retrieval of fear memories. Interestingly, impaired memory retrieval in limbic-kindled rats coincided with reduced expression of the neural activity markers Fos and Arc throughout the dentate gyrus. We also found that limbic-kindled rats had increased hippocampal neurogenesis as measured by BrdU cell counting, but almost none of these new neurons co-localized with Fos or Arc. In contrast, approximately 10% of mature adult-generated neurons co-localized with Fos in control and caudate-kindled rats. Collectively, our findings suggest that limbic, but not non-limbic kindling prevents the recruitment of mature adult-generated neurons into memory networks. These observations may help explain the cognitive impairments that are most commonly associated with limbic seizures.

Disclosures: **N. Nogovitsyn:** None. **J.J. Botterill:** None. **H.J. Caruncho:** None. **L.E. Kalynchuk:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

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Program#/Poster#: 461.07/LLL10

Topic: C.01. Brain Wellness and Aging

Support: UBACYT 20020130100258BA

UBACYT 20020130300033BA

PIP CONICET 00269

Title: Could changes in hippocampal neuron cytoarchitecture explain the depressive behavior of CB1 knockout mice?

Authors: ***H. A. BRUSCO**, D. SORIANO, F. CONDE, L. CALTANA;
IBCN (UBA-CONICET) Facultad De Medicina UBA, Buenos Aires, Argentina

Abstract: Cannabinoid receptors are expressed in the hippocampus. CB1R is preferentially expressed in pyramidal neurons and astrocytic processes, while CB2R is expressed in microglial, astroglial and endothelial cells.
CB1-receptor-knockout mice are used as an animal model of depression, although the morphological substrate of this behavior is not completely understood yet. And, as the hippocampus is an important area of the central nervous system involved in behavior, the aim of this work was to characterize hippocampal neurons in CB1-receptor-knockout mice.
The study was performed through immunofluorescence, Golgi, Western blot and electron microscopy techniques, focusing on the dentate gyrus and CA1 areas. The genetic ablation of CB1R produces an increase in the expression of CB2R, which suggests a compensatory regulation in the endocannabinoid system.
CB1-receptor-knockout mice evidenced a lower number of undifferentiated neuronal cells precursors (Nestin+ cells) and migrating neuroblasts (doublecortin+ cells). These data suggest that CB1-receptor-knockout mice suffer alterations in adult neurogenesis in the in dentate gyrus.
CB1-receptor-knockout mice also presented cytoskeletal alterations in hippocampal neurons, as evidenced by lower expression of Neurofilaments of 160Kda and 200Kda, as well as lower expression of MAP-2 protein. These results indicate that these neurons have scarce dendritic arborization and cannot correctly connect with their targets neurons.

The number of synaptic contacts studied by Golgi technique and electron microscopy did not show significant differences between CB1-receptor-knockout mice and wild type mice; however, synaptophysin expression was lower in CB1-receptor-knockout mice, probably suggesting alterations in synapse efficiency.
Taken together, these results could explain the behavior alterations in CB1-receptor-knockout mice.

Disclosures: **H.A. Brusco:** None. **D. Soriano:** None. **F. Conde:** None. **L. Caltana:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

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Program#/Poster#: 461.08/LLL11

Topic: H.01. Animal Cognition and Behavior

Support: NYSTEM C029157

NIMH R37 MH068542

Title: Imaging the functional integration of adult-born hippocampal granule cells into the dentate gyrus network

Authors: ***S. N. TUNCDEMIR**[1], G. TURI[2], G. ORDEK[1], A. LOSONCZY[2], R. HEN, 10016[3]; [1]Integrative Neurosci., RFMH Columbia Univ., New York, NY; [2]Neurosci., [3]Psychiatry, Neuroscience, Pharmacol., Columbia Univ., New York, NY

Abstract: The ability to discriminate among similar experiences is an important feature of episodic memory. The dentate gyrus of the hippocampus and its ability to incorporate newborn granule cells throughout life is crucial for the neural computation that generates spatial and contextual discrimination behaviors. In particular, preferential activation of 1-2-month old adult born granule cells (abGCs) within the predominantly silent dentate network, is hypothesized to allow abGCs to actively participate in these behaviors. Our lab has recently shown that abGCs exhibit higher firing rate and reduced spatial tuning with similar degree of spatial remapping between contexts compared to the mature GCs (mGCs) *in vivo* (Danielson NB. et al, 2016), suggesting that abGCs indirectly modulate the activity of the mGCs by recruiting local inhibitory networks (Drew LJ. et al, 2015). Nevertheless, circuit interactions of abGCs that contribute to information processing within the dentate gyrus during discrimination behaviors are poorly understood. To this end, we will use two-photon calcium imaging in awake behaving mice to monitor the activity of abGCs and mGCs over the 4-week period during which abGCs

functionally integrate into the dentate gyrus circuits. This preparation will highlight the emergence of spatiotemporal activity of abGCs during encoding of contextual information and will allow us to test how manipulating their activity affects the circuit dynamics of mGCs.

Disclosures: **S.N. Tuncdemir:** None. **G. Turi:** None. **G. Ordek:** None. **A. Losonczy:** None. **R. Hen:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 461.09/LLL12

Topic: H.01. Animal Cognition and Behavior

Support: MSFHR

NSERC

Title: The role of adult neurogenesis in visuo-spatial learning and memory is dependent on stress during training and sex.

Authors: ***T. P. O'LEARY**, D. ESPINUEVA, D. R. M. SEIB, J. S. SNYDER;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adult neurogenesis in the hippocampus is involved in visuo-spatial learning and memory, and also regulation of the hypothamic-pituitary-adrenal axis and stress-related behaviour. It is not yet clear, however, if the role of adult-born neurons in learning and memory is dependent on stress levels during the learning episode. To address this possibility, we used a transgenic rat that expresses the herpes simplex virus thymidine kinase, under the glial fibrillary acidic protein promoter (GFAP-TK). Administration of the anti-viral drug valganciclovir leads to death of mitotic neural precursor cells in the GFAP-TK rat. Beginning at 6 weeks of age, GFAP-TK rats were given valganciclovir orally for 6 weeks (twice per week), leading to a near-complete ablation of adult neurogenesis in the dentate gyrus. To assess the role of adult neurogenesis in learning and memory under stress, we tested GFAP-TK (N=96) and wild-type long-evans (N=103) rats in the Morris water maze using either cold 16°C water (high stress) or warm 25°C water (low to moderate stress). Rats completed acquisition training for 3 days (4 trials per day) with a probe trial (60 sec) on the following day to assess memory for the escape platform location. Both male and female rats were used, given that sex differences are found in behavioural responses to stress, and because few studies have examined sex differences in the role of adult neurogenesis. When trained in 16°C water, sex-dependent effects on learning were

found. In males, WT rats found the escape platform faster and travelled a shorter distance than GFAP-TK rats. The opposite result was found in females, as GFAP-TK rats located the escape platform faster and traveled a shorter distance than WT rats. These differences between genotypes were not found in 25°C water, and were not due to differences in swim-speed. In the probe trial, there was a trend for better memory performance in male WT rats than GFAP-TK rats, but only at the colder 16°C water. Overall sex differences in performance were found at 25°C but not 16°C, where male rats showed superior learning and memory, and slower swim speed compared to females. These results suggest that in males, adult neurogenesis is involved to a greater extent in visuo-spatial learning and memory during stressful learning episodes. The learning and memory performance of females, however, appears to be spared or even enhanced when neurogenesis is ablated. These results suggest a novel sex difference in the role of hippocampal adult-born neurons in visuo-spatial learning and memory, and underscore the importance of using both sexes when investigating the function of adult-born neurons.

Disclosures: T.P. O'Leary: None. **D. Espinueva:** None. **D.R.M. Seib:** None. **J.S. Snyder:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

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Program#/Poster#: 461.10/LLL13

Topic: H.01. Animal Cognition and Behavior

Support: NSERC CGSM

NSERC Discovery (to JSS)

NSERC Discovery (to JZ)

Title: Inhibition of adult neurogenesis impairs learning of spatiotemporal regularities

Authors: *R. YU, J. ZHAO, J. S. SNYDER;
The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The hippocampus is an important brain structure for learning in humans and other animals. Hippocampal neurogenesis is thought to play a critical role in pattern separation. Previous research has focused on the role of hippocampal neurogenesis in spatial learning, by training rodents to discriminate spatial contexts or locations. It is currently unknown what role hippocampal neurogenesis plays in the learning of regularities. Here, we examined how the inhibition of neurogenesis impacts learning of spatiotemporal regularities. Transgenic GFAP-TK

rats were used whose hippocampal neurogenesis was inhibited selectively in adulthood by valganciclovir. The GFAP-TK rats were compared with intact wild-type littermates. Rats were trained in a spatial water maze task where they were required to find a hidden escape platform in a tank of opaque water. The platform appeared in one of two possible locations. The rats were randomly assigned to and remained in one of the three conditions throughout training: the platform appeared repeatedly in the same location for all trials on a given day (simple regularities condition), alternated between the two locations on a trial-by-trial basis (complex regularities condition), or randomly appeared between the two locations on a trial-by-trial basis (no regularities condition). The swim trajectory and the latency to reach the platform were recorded for each trial and compared between the GFAP-TK rats (lacking neurogenesis) and the wild type rats (with neurogenesis). We found that when the platform location alternated on each trial, the GFAP-TK rats performed reliably worse than wild type rats, both in terms of latency to reach the platform and swim trajectory. However, when the platform appeared repeatedly in the same location, or randomly appeared between locations, the GFAP-TK rats were not different from wild type rats. These results suggest that neurogenesis is critical for learning of complex but not simple spatiotemporal regularities.

Disclosures: **R. Yu:** None. **J. Zhao:** None. **J.S. Snyder:** None.

Poster

461. Adult Hippocampal Neurogenesis: Consequences of Altering Neuronal Production

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 461.11/LLL14

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: The effects of amygdala kindling on hippocampal neurogenesis and pattern separation.

Authors: ***J. K. CARR**, H. LEHMANN, N. M. FOURNIER;
Trent Univ., Peterborough, ON, Canada

Abstract: Pattern separation is a neural computational process involving the dentate gyrus (DG), which is thought to underlie critical aspects of memory retention such as maintaining independence of different memory representations. Recent evidence has indicated that young adult-generated dentate granule cells (GCs) mediate pattern separation (i.e. behavioural indices of pattern separation are impaired by down-regulation of neurogenesis and enhanced by up-regulation). In addition, hippocampal neurogenesis is also substantially altered by amygdala kindling, and these alterations are thought to underlie the progressive development of hyper-

excitable circuitry and chronic seizures seen in temporal lobe epilepsy. Specifically, amygdala kindling causes a dramatic increase in the rate of neurogenesis in the short-term, followed by a long-term suppression of neurogenesis which often falls below baseline levels. These changes in the rate of cell proliferation also coincide with aberrant changes in the migration, excitability, and functional integration of these young GCs into existing hippocampal circuits. These two lines of evidence converge on an interesting question: What impact does amygdala kindling have on pattern separation? The aim of our study was to characterize alterations in pattern separation in rats following short- (30 stimulations) and long-term amygdala kindling (99 stimulations) using a context discrimination task, which relies on pattern separation and is sensitive to manipulations of hippocampal neurogenesis. Bromodeoxyuridine (BrdU) was injected in short-term and long-term kindled rats 2-weeks prior to behavioral testing in order to label proliferating neurons, and post-mortem analysis was conducted on the brains using BrdU and IEGs (c-Fos and zif268) immunohistochemistry in order to examine the recruitment of young GCs into the network supporting pattern separation. Our findings suggest that chronic seizures may disrupt the participation of adult-born neurons in hippocampal networks involved in cognitive and mnemonic function. Support: NSERC Theme and Topic 1. H.01.n. Learning and Memory: Hippocampal Circuits Theme and Topic 2: B11.g. Animal Models Key words: Neurogenesis; Learning and Memory; Seizure

Disclosures: **J.K. Carr:** None. **H. Lehmann:** None. **N.M. Fournier:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Program#/Poster#: 462.01/LLL15

Topic: H.01. Animal Cognition and Behavior

Support: Canadian Institutes of Health Research

Natural Sciences and Engineering Research Council

Title: Modulation of glutamatergic neurons in the medial septum in the freely moving mouse

Authors: *J. ROBINSON[1,2], F. MANSEAU[1,2], S. WILLIAMS[1,2];
[1]Inst. Universitaire En Santé Mentale Douglas, Verdun, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Neurons in the medial septum diagonal band of Broca (MS-DBB) provide important connections to the hippocampus and are critical for spatial learning and memory. Three main

neuronal populations have been identified in this region: cholinergic, GABAergic and glutamatergic. It has previously been reported that glutamatergic neurons provide connections within the MS-DBB onto both GABAergic and cholinergic neurons as well as providing sparse connections to the hippocampus. In addition, it was recently shown that optogenetic activation of glutamatergic neurons can powerfully drive CA1 hippocampal theta rhythms and initiate locomotion. To further explore the role of MS-DBB glutamatergic cells we have used optogenetics to specifically modulate this population in the freely moving mouse. Here, glutamatergic MS-DBB neurons were targeted through injection of a Cre-dependent AAV viruses containing the light-sensitive proteins, ChETA or ArchT constructs, that were used to activate or suppress glutamatergic neurons in a VGLUT2-CRE mouse line. Using this model we aim to determine how modulation of glutamatergic neurons from the MS-DBB affects theta rhythms across the hippocampus and influence animal behavior. In the freely moving mouse, we have observed that activation of these neurons drive theta rhythms across the hippocampal formation. In contrast, suppression of these glutamatergic neurons has little influence on theta power or frequency. Finally, we will examine the effects of activation and suppression of this population on exploration in an open field setting. These experiments will help to determine the role glutamatergic MS-DBB neurons play in freely behaving mice.

Disclosures: **J. Robinson:** None. **F. Manseau:** None. **S. Williams:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Topic: H.01. Animal Cognition and Behavior

Support: J.-B.B. is supported by a postdoctoral fellow from the Fyssen Foundation (France)

Title: Calcium imaging of medial septal neurons in freely-behaving mice

Authors: ***J.-B. BOTT**, S. WILLIAMS;
Dept of Pyschiatry, McGill University, Douglas Ins, Verdun, QC, Canada

Abstract: Medial septal neuronal population are GABAergic, glutamatergic and cholinergic. The medial septum is known for modulating the activity in the hippocampal formation, but the contribution of glutamatergic and cholinergic neurons remain poorly understood. More specifically the spontaneous activity pattern of those neuronal populations during behavior has not been well characterized as recording identified neuronal subpopulation in vivo in freely-moving mice is challenging.

Using calcium imaging, our objective is to characterize the spontaneous activity of cholinergic and glutamatergic neurons during various behavior in freely-moving mice. VGLUT2-Cre and ChAT-Cre mice were injected with 0.5µl of the Cre-dependent *AAV2/9-Syn-Flex-GcAMP6f* virus. Three weeks later, mice were implanted with a chronic imaging device and subjected to different behavioral tasks.
The activity pattern of glutamatergic and cholinergic neurons from the medial septum differed and appeared highly specific to different behavioral aspects of the task. More specifically, the glutamatergic neurons elicited a locomotion-related behavior whereas cholinergic cells displayed a pattern of activity that suggested a memory-encoding involvement.
Those results suggest that medial septum glutamatergic and cholinergic subpopulations contribute differentially to hippocampal-dependent cognition. Moreover, calcium imaging appears particularly adapted to extract sub-population specific activity patterns in relation to the animal behavior.

Disclosures: J. Bott: None. **S. Williams:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 462.03/LLL17

Topic: H.01. Animal Cognition and Behavior

Support: Brain Canada

Title: Spatial reference memory impairments are associated with abolished CA1 theta-gamma cross-frequency coupling in freely behaving J20 APP mice

Authors: *G. ETTER[1,2], S. WILLIAMS[1,2];
[1]Douglas Mental Hlth. Inst., Verdun, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Alzheimer's disease (AD) has been associated with amyloid beta (Ab) aggregation, subsequent hippocampal neurodegeneration and memory defects. The exact nature and chronology of these pathological events remains largely unknown. Theta-gamma cross-frequency coupling (CFC), a physiological phenomenon that has been associated with memory encoding and retrieval, has been previously shown to be decreased in complete hippocampus preparations from 3 weeks old transgenic AD mice model. Several authors have proposed to segregate gamma band oscillations into slow (30-60 Hz), and fast (60-120 Hz) clusters that may rely on CA3 and medial entorhinal cortex inputs, respectively. In the present study, we have monitored CA1 local field potentials in the freely behaving J20 AD mice model (PDGF-APPSw,

Ind) trained to seek a reward on a modified appetitive version of the Barnes maze as well as during REM sleep. At 6 months of age, J20 mice display more spatial errors as well as non-targeted exploration during the probe trial compared to non-transgenic (NTg) counterparts. Using a standardized measure of CFC, we show that theta-gamma CFC is significantly reduced during REM sleep, while it is abolished (not significantly different from a random distribution) when mice actively explore the Barnes maze. When isolating gamma oscillations using wavelet convolution, we show that NTg mice display dynamics (predominant fast gamma before reaching the target, followed by predominant slow gamma) that are abolished in J20 mice. This study suggests that circuits underlying cross-frequency coupling are affected very early in AD mice models and might underlie the spatial memory defects and suggests that the CFC may be an early biomarker of AD.

Disclosures: G. Etter: None. **S. Williams:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Topic: H.01. Animal Cognition and Behavior

Support: Alzheimer Society of Canada

Brain Canada

Title: Deep Brain Stimulation to improve memory function in an animal model of Alzheimer Disease

Authors: *E. VICO VARELA, S. WILLIAMS;
McGill Univ. -Douglas Mental Hlth. Uni. Inst., Montreal, QC, Canada

Abstract: Deep Brain Stimulation (DBS) is a therapeutic approach being evaluated for the treatment of Alzheimer Disease (AD). AD is a neurodegenerative disorder, presenting a dysfunction in the 'memory network': structures implicated in memory, such as the hippocampus and entorhinal cortex, are affected earliest and most severely. The current study aims to identify memory-facilitation mechanisms of fornix DBS in a transgenic mice model of AD (J20) by examining electrophysiological recordings of the CA1 hippocampal region. Recordings of the CA1 region were collected in 3 months old mice during REM, before and 24 hours after the Passive Avoidance task and while they were exploring objects during a Novel Place Object task. We show that transgenic positive mice display significant impairment in memory performance in

the Passive Avoidance task as measured by the latency to enter the dark chamber and Novel Place Object, assessed by the Recognition Index. Applying chronic Theta-Burst fornix DBS during the 24 hours after the initial learning of Passive Avoidance and during 3.5 hours after the Novel Place Object first exposure to the objects had a rescuing effect on the memory impairment. We intend to examine the relationship between the stimulation, behaviour and theta oscillations in the hippocampus to elucidate the modulatory effect of fornix DBS on the memory network.

Disclosures: **E. Vico Varela:** None. **S. Williams:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Topic: H.01. Animal Cognition and Behavior

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Fondation Jerome Lejeune Grant

Spain Ramon y Cajal Grant RYC-2012-10042

Title: Altered prefronto-hippocampal neural network dynamics in a murine model of Down syndrome during memory processing

Authors: M. ALEMANY, T. GENER, *M. PUIG;
IMIM -Hospital del Mar Med. Res. Inst., Barcelona, Spain

Abstract: Alterations in neural activity in the prefrontal cortex (PFC) and hippocampus (HPC) have been postulated as pathophysiological mechanisms underlying learning and memory deficits in mental disorders. However, it is still unclear if PFC-HPC miscommunication is behind cognitive impairment in intellectual disability. Down Syndrome (DS) is the most common genetic cause of intellectual disability and is modeled in Ts65Dn mice by a partial trisomy of the murine equivalent of human chromosome 21. Ts65Dn mice exhibit dendritic and synaptic abnormalities in the PFC and HPC and severe cognitive impairments in PFC- and HPC-dependent tasks. However, whether these cellular alterations affect neural network activity and PFC-HPC communication is still unknown. Interestingly, most of these DS phenotypes are rescued both in DS human subjects and Ts65Dn mice by Epigallocatechin-3-gallate (EGCG), the main component of green tea and potent inhibitor of DYRK1A, a serine/threonine kinase whose gene is triplicated in trisomy 21. Thus, it is of high translational value to understand the cellular

mechanisms underlying EGCG-mediated cognitive amelioration. To tackle this question, we have recorded neural network activity in the PFC and HPC of Ts65Dn mice performing the Novel Object Recognition (NOR) task before and after treatment with EGCG.
METHODS: Local field potentials were recorded in the PFC and HPC of freely-moving mice via tungsten stereotrodes with the Open Ephys data acquisition system during quiet wake, open field exploration, and the performance of the NOR task that evaluates short- (1h) and long-term (24h) memory. Ts65Dn mice and their wild-type littermates were treated orally with EGCG for one month and neural activity was recorded before, during and after the treatment.
RESULTS: Our preliminary results indicate that Ts65Dn mice show alterations in neural network dynamics affecting several frequency bands in both PFC and HPC and in PFC-HPC synchrony compared to controls. Particularly, Ts65Dn mice show decreased theta oscillations in the PFC but increased theta and gamma in the HPC. Moreover, PFC-HPC synchrony is increased at theta but decreased at beta and gamma frequencies. Importantly, one month of treatment with EGCG partly rescued these oscillatory alterations in the Ts65Dn mice, especially in the HPC. Ongoing analyses will determine the relevance of these effects for memory.
CONCLUSIONS: Our findings suggest that alterations in neural network dynamics in PFC and HPC and in PFC-HPC synchrony may underlie cognitive impairment in Ts65Dn mice. EGCG may ameliorate cognitive deficits both in DS subjects and Ts65Dn mice via its effect on the PFC-HPC neural network.

Disclosures: **M. Alemany:** None. **T. Gener:** None. **M. Puig:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: 2013 NARSAD Young Investigator Grant

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Title: Serotonin 5-HT1A and 5-HT2A receptors and antipsychotics modulate gamma and theta rhythms and prefronto-hippocampal connectivity in behaving mice

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Abstract: Patients with schizophrenia (SCZ) display cognitive impairment that correlates with alterations in brain oscillations. For example, gamma oscillations in frontal areas are reduced in SCZ patients relative to healthy subjects during the performance of executive tasks. Moreover, genetic murine models of SCZ suggest that an abnormal synchrony between the prefrontal cortex (PFC) and the hippocampus (HPC) may also account for the observed cognitive impairment. The main goal of this study is to determine the neural substrates of cognitive amelioration mediated by atypical antipsychotic drugs. Specifically, we have investigated the role of the serotonin system in the beneficial actions of atypical vs. typical antipsychotics on cognition. To do so, we have recorded neural oscillatory activity in the PFC and HPC of freely-moving mice performing a memory test while administering pharmacological agents and antipsychotics that activate or inhibit serotonin 5-HT1AR and 5-HT2AR. We report drug actions on PFC-HPC oscillatory activity and how this may account for memory amelioration mediated by atypical antipsychotic drugs.
METHODS: Local field potentials were recorded in the PFC and HPC of freely-moving mice via tungsten stereotrodes with the Open Ephys data acquisition system during 1) the exploration of an open field and 2) the performance of the novel object recognition test that evaluates short- (1h) and long-term (24h) memory. The following serotonin and antipsychotic drugs were administered i.p.: 5-HT1AR agonist 8-OH-DPAT, antagonist WAY100635; 5-HT2AR agonist DOI, antagonist M100907; atypical antipsychotics clozapine, risperidone; typical antipsychotic haloperidol.
RESULTS: Our results show that 5-HT1AR activation with 8-OH-DPAT markedly reduces theta and gamma oscillations in the PFC and HPC, an effect reversed by WAY100635. Similar results were observed with clozapine, risperidone and haloperidol along with an exacerbation of delta waves; however, this was not reversed by WAY100635. This suggests that antipsychotic actions on PFC-HPC oscillations may not be mediated by 5-HT1AR during alert states, a result unexpected for clozapine. Moreover, 8-OH-DPAT increased PFC-HPC functional connectivity (Pearson´s correlation) at gamma ranges. M100907 also reduced theta and gamma PFC-HPC oscillations and exacerbated delta waves. Ongoing analyses will determine the relevance of these effects for memory.
CONCLUSIONS: 5-HT1AR and 5-HT2AR in the PFC and HPC exert strong influences on gamma and theta oscillations and PFC-HPC connectivity. However, their involvement in antipsychotic-mediated effects needs further elucidation.

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Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Topic: H.01. Animal Cognition and Behavior

Title: Quantitative EEG as a tool for monitoring efficacy of putative cognitive enhancers: Preclinical investigation

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Abstract: Quantitative electroencephalography (qEEG) is an important tool in quantifying changes in the brain activity. In animal models of cognition, synchronous EEG activity observed in a frequency range of 4 to 8 Hz (Theta) and 30 to 50 Hz (gamma) has been associated with cognitive enhancement. Neurological diseases such as Alzheimer's disease (AD) often affect the complex neuronal network by degeneration of neurons, resulting in a "slowing" of the EEG signal which corresponds to increase of low-frequency spectral power bands and a decrease of higher-frequency bands. If these theta and gamma oscillations are associated with mnemonic or cognitive function, qEEG can be used a potential biomarker for the cognition-enhancing drugs regardless of their primary biological target.
Theta modulation studies were conducted in urethane anesthetized rats and mice. Electrical stimulations were applied to the nucleus pontis oralis and power of oscillatory activity was measured in hippocampus CA1 region. Modulation in oscillatory gamma activity in freely moving animals was measured using telemetric device (Data Sciences International). In the current study, we evaluated several drugs that were shown to have effect on the cognition in preclinical behavioral models.
Acetylcholinesterase inhibitor, donepezil, and muscarinic receptor selective positive allosteric modulator induced increases in the low frequency bands like theta in anesthetized animals and gamma oscillatory power in freely moving animals. The nonselective muscarinic acetylcholine receptor antagonist, scopolamine decreased the power of theta and the gamma oscillatory power in anesthetized and freely moving animals, respectively.
These results support that qEEG could be an effective biomarker for the evaluation of potential putative cognitive enhancers in the treatment of Alzheimer's disease.

Disclosures: **S. Daripelli:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **V. Benade:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **G. Ayyanki:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD,

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Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Title: Impairments in spatial memory representations in freely moving 3xTg mice

Authors: *A. J. MABLY, D. T. JONES, B. J. GEREKE, L. L. COLGIN;
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Abstract: Spatial memory impairments are both a clinical feature of Alzheimer's disease (AD) and a characteristic common to many mouse models of AD. The hippocampal formation, and particularly hippocampal place cells, are thought to be essential for correct cognitive representation of spatial information. Consequently, it could be speculated that improper formation of place cells' spatial receptive fields (place fields), or instability of these fields over time contributes to the spatial memory impairments seen in AD. To assess whether place cell activity was aberrant in an AD mouse model, we recorded from hippocampal subfield CA1 of 3xTg and wildtype (Wt) mice whilst they traversed a familiar circular track. Mice ran three 10 minute sessions a day and place fields were compared across trials within each day. Place field locations and firing rates were significantly less stable across sessions in 3xTg mice compared to Wt mice. In addition, place cells in 3xTg had lower spatial information content than those of Wt mice. Furthermore, rhythmic coordination of place cell spiking was altered in 3xTg mice. These results indicate a disruption in the cognitive representation of space in an AD mouse model and, with further investigation, may provide insights into the cellular mechanisms of spatial memory impairments in AD.

Disclosures: A.J. Mably: None. **D.T. Jones:** None. **B.J. Gereke:** None. **L.L. Colgin:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Klingenstein Fun

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Title: Experience-dependent trends in the CA1 cross spectrum revealed by a generalized additive mixed model

Authors: *B. J. GEREKE[1], A. J. MABLY[2], L. L. COLGIN[2];
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Abstract: Understanding how coordinated interactions among neurons represent information that supports learning, memory, and behavior is a central goal of neuroscience. Insight into these interactions can be gained by studying both spiking neurons as well as the local field potential (LFP), which is usually taken as a signature of neuronal population activity. Spiking data is commonly studied using multivariate regression methods, such as the generalized linear model (GLM). These models have had success identifying the relative importance of covariates associated with spiking such as sensory stimuli, animal behavior, intrinsic neuronal properties, and the spiking of other neurons. Despite their popularity with spike train data, however, applications of multivariate regression methods to LFP data remain surprisingly scarce. Here, we employ a generalized additive mixed model (GAMM), a nonparametric extension of the GLM, to study the LFP cross spectrum of hippocampal area CA1. The model is optimized for large data sets, typical of LFP data, and successfully identifies the relative importance of documented frequency-specific covariate effects (e.g., running speed, theta phase, etc.), along with their second-order interactions, while accounting for “random effects” that may be attributed to a particular animal and/or recording session. The GAMM framework also supports the investigation of temporal trends, which can be difficult to study using univariate methods. We identify how such trends change in a frequency-specific manner with a rodent’s experience running on a 1-dimensional track. Recordings were obtained as animals ran on the track three sessions per day for ~5-7 days. During the first few minutes of the second and third sessions of

each day, theta (6-12 Hz), slow gamma (25-55 Hz), and fast gamma (55-100 Hz) rhythms were all transiently elevated. However, in the first session of each day, this increase in slow gamma did not occur, whereas the increase in theta was stronger than in other sessions. Such experience-dependent changes in the LFP may have interesting implications for place field backward expansion, which displays similar trends (Mehta et al., PNAS, 94, 1997), as well as for memory in general.

Disclosures: B.J. Gereke: None. **A.J. Mably:** None. **L.L. Colgin:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Title: The correlation between gamma frequency and running speed in the dentate gyrus and CA2 of freely behaving rats

Authors: *C. ZHENG[1,2], Y.-T. HSIAO[1,2], L. L. COLGIN[1,2];
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Abstract: Slow (~25-55 Hz) and fast (~60-100 Hz) gamma rhythms are thought to represent distinct network processing states in the entorhinal-hippocampal network. Although gamma is maximal in the dentate gyrus (DG) region (Bragin et al., *J Neurosci* 1995), few to no studies have investigated slow and fast gamma rhythms in DG. Moreover, recent studies of place cells in CA2 (e.g., Mankin et al., *Neuron* 2015) have emphasized the importance of analyzing electrophysiological signals from CA2 separately from CA3 and CA1. Although little to nothing is known about gamma rhythms in CA2, a reasonable hypothesis is that gamma in CA2 resembles gamma in CA3, considering that CA3 projects to CA2. Here, we examined slow and fast gamma rhythms in DG (n = 4 rats) and CA2 (n = 3 rats). We focused our investigation on the relationship between running speed and gamma frequency in DG and CA2. Previous work has shown that frequencies of slow and fast gamma rhythms in hippocampal subfields CA1 and CA3 show different correlations with running speed (Zheng et al., *Hippocampus* 2015). Slow gamma frequencies do not change much with running speed, whereas fast gamma frequencies increase significantly as a function of running speed. New results from DG were similar to

previous CA3 results. Specifically, DG slow gamma power was maximal at relatively low running speeds, and slow gamma frequencies did not change much with running speed. Also, as in CA3, DG fast gamma frequencies increased with increasing running speed, and DG fast gamma power was low when rats ran at speeds exceeding ~25 cm/s. As was the case in other hippocampal subfields, CA2 slow gamma power was maximal at relatively low running speeds, and CA2 slow gamma frequencies were not strongly affected by running speed. However, in contrast to DG and CA3, CA2 power in the fast gamma range strongly increased at running speeds greater than ~25 cm/s. These results suggest that slow and fast gamma in DG and CA3 may be functionally similar and additionally reveal another electrophysiological feature that distinguishes CA2 from other hippocampal subfields.

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Poster

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Kavli Institute for Brain and Mind IRG

Title: Social investigation of conspecifics and robots: oscillatory neural dynamics

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Abstract: Social interactions are a critical feature of most animals' lives, and are key to learning and survival. Rodents show many types of social behaviors including following, mirroring, wrestling, and sniffing. Rats reliably show selectivity in social behavior, behaving differently toward novel or familiar conspecifics. Recent studies have shown that rats will also engage in complex social behaviors with robotic social partners. Here we attempt to shed light on the brain signatures underlying social recognition, whether to a conspecific or a robot. Social recognition is a complex chemosensory process that can be empirically studied by habituation-dishabituation paradigms. These paradigms have reliably shown that rats will persistently investigate a novel conspecific for longer periods of time than a familiar conspecific. After being habituated to a conspecific, each rat was presented with two enclosures containing either a familiar conspecific

or a novel one. A second condition included repeating the task, using novel and familiar robots. A control condition included an additional repetition of the task using novel and familiar objects. Local field potentials were recorded from an anatomical circuit in the rat that is critical for social processing: main olfactory bulb (MOB), CA2 subregion of the hippocampus, and medial amygdala (meA). These regions have been associated with social recognition, predator identification, sexual behavior, and processing odor signals. Our recordings investigated oscillatory phase coupling in this circuit during social investigation, as a means of understanding the circuit dynamics that support social recognition. Characteristic patterns emerged, indicating that engaging in the social recognition task recruits the circuit. For example, theta phase coupled gamma oscillations in MOB show a significant increase in amplitude during novel social investigation. There are also identifiable differences of phase coherence between theta oscillations in MOB, meA, and CA2 during social investigation of conspecific, robot, and object. In addition to the primary findings, this study demonstrates how robots can be used as a feasible control to help isolate socially relevant neural dynamics.

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Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Support: NSF 0910485

Title: Transformation of independent oscillatory inputs into temporally precise rate codes.

Authors: ***D. A. NITZ**[1], D. TINGLEY[2], A. ALEXANDER[3], L. QUINN[3], A. CHIBA[3];
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[3]Cognitive Sci., UCSD, La Jolla, CA

Abstract: The basal forebrain's afferent and efferent structure suggests a capacity for mediating temporal coordination of activity in functionally distinct brain regions during the execution of complex behavioral sequences. To examine the role of basal forebrain (BF) in such a process, we recorded local field potentials and multiple single neuron spiking activity in the ventral pallidal and substantia innominata sub-regions of basal forebrain during performance of a selective attention task. Local field potentials provided a measure of the temporal organization of basal forebrain inputs while spiking activity patterns among basal forebrain ensembles provided a

measure of its output. Both types of neural signals were examined for their dynamics across the full set of task epochs. In addition, analyses specifically addressed the relationships between local field potential and spiking dynamics in order to detect potential input/output transformations of the basal forebrain. BF field potentials were dominated by four amplitude-independent oscillations. The higher frequency oscillations, beta (20-35 Hz), gamma (45-65 Hz), and hi-gamma (80-150 Hz) occurred as transient events and were temporally organized by the phase of the slowest, a theta rhythm (5-9 Hz). The latter finding strongly suggests that the BF theta rhythm may be responsible for sequencing higher frequency transients through time. Both features of the local field potential are consistent with time-division and frequency-division models for decoding incoming multiplexed signals. Finally, oscillations in each frequency range exhibited robust changes in amplitude as a function of task epoch. Spiking activity for a large sub-population of BF neurons was robustly phase locked to one or more of the observed LFP frequency bands. As shown in prior work, spike rates for individual neurons were strongly correlated to one or more specific task epochs and the population as a whole produce a ensemble firing rate vector for each task epoch. Trial-to-trial variation in oscillatory amplitudes were strongly correlated to spike rates of individual neurons identifying a mechanism by which oscillatory inputs can be transformed into spike rate codes. Finally, similar to observations for hippocampal place cells, spiking activity across task epochs precessed in phase against the ongoing theta-frequency local field potential oscillation. Together, the findings reveal a process by which associative brain regions such as BF can integrate independent oscillatory inputs and transform them into sequence-specific, rate-coded outputs that are adaptive to the pace with which organisms interact with their environment.

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Poster

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Support: NS054281

Title: Small conduction delays induce global synchrony in sparsely but strongly connected inhibitory networks.

Authors: *C. C. CANAVIER[1], R. A. TIKIDJI-HAMBURYAN[2];
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Abstract: Inhibitory interneuronal networks have been shown to mediate gamma oscillations that in turn are hypothesized to underlie many cognitive functions. We simulated an inhibitory network of 300 Hodgkin-Huxley neurons using the simulation package NEURON to successfully test the predictions of phase resetting theory under the assumption that the coupling is pulsatile but not necessarily weak. We extended our previous analysis of synchrony and antiphase in two neuron networks with conduction delays to global synchrony and a two-cluster mode in large all-to-all coupled networks. We generated the phase resetting curve (PRC) of a single self-connected neuron with delayed feedback equal to half the connections in the network, and using a perturbation equal to the IPSP received from the other half of the connections in the network. Using these PRCs, we analyzed a two-cluster analog of the network and show that in cases of bistability between global synchrony and a two-cluster mode, phase resetting theory under pulsatile coupling assumptions can predict the basins of attraction that determine which mode is dominant. The results are valid even in cases in which the individual interneurons are not spontaneous pacemakers, but display post-inhibitory rebound (PIR) that supports a network oscillation that is self-sustaining once it has been initiated by a strong inhibitory input. Strong inhibition that induces PIR generally results in a monotonically increasing PRC with a sharp discontinuity at phases of 0 and 1. This discontinuity destabilizes global synchrony at zero delay, but small delays stabilize synchrony as long as the slope of the PRC is less than one. These delays also tend to prevent cluster solutions; we present the criteria for the minimum delay that guarantees global synchrony for this PRC shape. We further extend these methods from all-to-all to sparsely coupled networks by reducing the strength of the self-connection and the PRC-generating perturbation in the two neuron analog of the network. We then show that in the presence of the jitter in spike timing introduced by sparse connectivity, attractors must have a minimum width in the parameter space of conduction delays in order to be robustly observed.

Disclosures: C.C. Canavier: None. **R.A. Tikidji-Hamburyan:** None.

Poster

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Title: Mechanisms of spike timing in a detailed computer model of a medial entorhinal cortical stellate cell

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Abstract: Stellate cells of the rat medial entorhinal cortex are known to exhibit distinct spike timing in response to patterned inputs, a characteristic likely important for coding position in putative grid cells. For example, when receiving input sufficient to observe the membrane potential theta-ramp oscillation associated with spatial navigation, the spike timing appears more consistently related to the theta phase of membrane potential oscillation than the voltage at which the spike is initiated (Domnisoru et al, 2013, Figure 4a). The extent to which this spike timing is influenced by intrinsic properties of the stellate cell versus phasic network inputs is unknown. Here we explore, using a detailed, biologically constrained computational model of a medial entorhinal cortical stellate cell, the extent to which various intrinsic properties and afferent inputs can influence the timing of stellate cell spikes during the theta-ramp regime. Our stellate cell model contains several ion channel types including HCN current, as well as detailed dendritic morphology and realistic synaptic inputs (Giocomo and Hasselmo, 2008; Heys et al, 2010, 2012; Pastoll et al 2012; Tsuno et al, 2015; Shay et al, 2015; Ferrante et al, 2016). We first validate our stellate cell model, showing its relevance to biological stellate cells in a variety of conditions, before systematically manipulating the stellate cell model to determine the dominant mechanisms that control its spike timing. The results of the simulations suggest an important role for subthreshold, intrinsic currents in setting the spike timing of stellate cells during spatial navigation. These results may guide future in vitro and in vivo experiments and can also inform future computational network models of grid cell formation.

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Poster

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Title: State dependence of directional interactions between basal forebrain and visual cortex in theta and gamma band local field potentials

Authors: *A.-L. KLAASSEN[1], J. NAIR[1], J. POIROT[1], A. VYSSOTSKI[2], B. RASCH[1], G. RAINER[1];
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Abstract: The basal forebrain (BF) is an important regulator of cortical excitability and responsivity to sensory stimuli, and plays a major role in wake-sleep regulation. While the influence of the BF on EEG or LFP signals in the cortex has been extensively documented, relatively little is known about local field potential (LFP) activations within the BF.
Here we describe endogenous LFP oscillations in the basal forebrain during wakefulness, REM and SWS states that were recorded using a miniature wireless device during these different behavioral states while animals were in their home cage. Based on bilateral recordings from BF and the visual cortex (VC), we performed coherence and Granger causality analyses to study behavioral state dependent directional influences between the BF and visual cortex (VC).
We found robust gamma activity particularly during wakefulness, as well as to a lesser extent during SWS and REM. In each of the behavioral states there were robust peaks in the gamma band, each with a distinct peak oscillation frequency. Whereas gamma band directional impact of VC on the BF was negligable, we observed a pronounced directional influence of BF on the VC during wakefulness and SWS. The opposite was the case in the theta band, where we observed bidirectional influence between the two brain regions with a predominant component from VC onto the BF during REM and wakefulness.
Our evidence suggests that while gamma band effects were regulated specifically within each brain hemisphere, theta band activations were coordinated globally across both hemispheres. We report novel aspects of endogenous BF LFP oscillations and their relationship to cortical LFP signals during sleep and wakefulness. We link our findings to known aspects of the heterogeneous BF networks underlying the LFP activations, show that the Granger causality analyses can faithfully recapitulate many known attributes of these networks.

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Poster

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Title: Decoding recalled color imagery using ECoG signals in the macaque inferior temporal and prefrontal cortices

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Abstract: Decoding mental content from brain activity is one of the challenging goals in neuroscience. In this study, we attempted to decode recalled color imagery using ECoG signals in the macaque cerebral cortex. We implanted 128- and 64-channel ECoG electrode arrays with a 2.5-mm spaced grid configuration on the inferior temporal and prefrontal cortices (ITC and PFC), respectively, which are known to be important for memory recall. Two monkeys were trained to perform a color recall task. In the task, an achromatic cue stimulus was presented, and after an interval, 1-2 colored shapes were sequentially presented as choice stimuli. The monkeys were required to release a lever upon the presentation of correctly colored choice that was associated with the cue, to receive a reward. We used two different types of cues: (1) scene images, and (2) Fourier descriptors. The monkeys were supposed to recall and maintain the color imagery until the choice behavior. Using the spectral powers of ECoG signals in different frequency bands as input features, we constructed a linear support vector machine classifier to predict the presented cue on a trial-by-trial basis. We found that the classifiers successfully predicted the cue from ECoG powers either in the ITC or PFC. Theta-band (4-8 Hz) powers provided the best classification performance. Next, to examine the classifier performance for recalled color imagery, but not for cue information, we calculated the generalization performance of classifiers across tasks using different cues: we trained a classifier on the data set from tasks using one type of cues (scene images) and tested them on a data set from tasks using another type of cues (Fourier descriptors) and vice versa. The result showed that even across tasks using different cues, the classifiers, constructed from theta-band powers in the PFC during or just after the cue presentation, successfully predicted the monkey's color choice. However, the classifiers

from ECoG signals in the ITC failed to predict the color choice. Because the successful classification from ECoG signals in the PFC was enabled prior to the monkey's choice behavior, this classification should be based on the pattern of ECoG signals specific to the recalled color imagery. A cortical surface-based searchlight decoding approach revealed that the channels with higher classification performance in the theta-band powers were localized in the focal regions in the PFC. Our results demonstrated that ECoG recoding potentially provides a method to read out memory content.

Disclosures: **H. Tanigawa:** None. **K. Majima:** None. **R. Takei:** None. **K. Kawasaki:** None. **H. Sawahata:** None. **K. Nakahara:** None. **A. Iijima:** None. **T. Suzuki:** None. **Y. Kamitani:** None. **I. Hasegawa:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 462.17/LLL31

Topic: H.01. Animal Cognition and Behavior

Support: R01MH065658

Title: Fimbria fornix stimulation parameters determine oscillations coupling of the prefrontal cortex and hippocampus

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Abstract: Specific patterns of neural oscillations are associated with many cognitive processes, including learning and memory, and the synchrony of oscillations among different brain regions may represent signaling and communication between those regions. Alterations in oscillations in the theta and gamma frequency bands have been linked to memory impairment and dysfunction in brain diseases and disorders such as Alzheimer's and schizophrenia. Different studies report conflicting results, so that the same electrical stimulation protocol can either improve or impair memory performance in people and animal models including rats. We hypothesized that stimulation parameters even within the same operationally defined category, e.g. theta burst stimulation, alter oscillation patterns across structures that ultimately determine their functional effects. To test this hypothesis, we varied the magnitude and temporal patterning of fimbria fornix (FFx) electrical stimulation in behaving rats. Stimulation electrodes were implanted in the FFx and recording electrodes were placed in both the medial prefrontal cortex (mPFC) and hippocampus. We varied the duration, frequency, and amplitude of current delivery and analyzed

local field potentials (LFPs) before, during, and after stimulation using custom-written Matlab (MathWorks) code and the Chronux toolbox (www.chronux.org). Preliminary results show that FFx stimulation modulated the amplitude and phase relationship of theta LFPs (4-12 Hz) in CA1 and mPFC, and that they varied with stimulus type, duration, and magnitude. Strong theta burst stimulation (>300 uV) increased the synchrony and reduced the phase differences between mPFC and hippocampal theta LFPs. Future experiments will vary patterned stimulation to determine how different stimulation parameters alter cognitive performance in memory tasks.

Disclosures: V. Luo: None. **M.L. Shapiro:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Support: JSPS KAKENHI（24223004, 24243069, 26115513)(Y. S.)

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JSPS KAKENHI (11J07301)(T. N.)

SCOPE, MIC(152107008)(S. T.)

Title: Learning stages in a rule switching task affects theta-gamma couplings in rat hippocampus

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Abstract: Hippocampal oscillations, particularly theta (6-12 Hz) and gamma (30-100 Hz) bands, play important roles in several cognitive functions. Theta and gamma oscillations show cross-frequency coupling (CFC), and the theta rhythm phase can modulate the amplitude of gamma oscillations. This phase-amplitude modulation is thought to have several cognitive roles, e.g., sensory signal detection, attention and memory processes. Previous studies found that the strength of CFC correlated with task performance during learning. To explore this correlation in more detail, we recorded local field potentials from the hippocampus of rats while they were performing a rule-switching task. In the task, rats had to choose a correct hole by using presented cues. Two types of cues (light and tone) were presented at the same time, however only one type of the cues was valid, and the other was distractor. The cue type indicating correct holes was

fixed through a session and we call such valid cue type "rule" in this task. Once the rats had learned the rule and their performance reached the criterion, the rule was changed to the other and the rats should learn the new one. The modulation index (MI), an index of the strength of CFC, showed event related changes during the rats was performing in learning trials. However these changes did not appear in control (no learning) trials. This result suggests that the coupling between theta and gamma bands in the hippocampus is engaged in rule learning. Next, we examined changes of MI through stages of learning. Our data suggested that the coupling between theta and high-gamma oscillations increased in early learning stage, but such change did not occur in late learning stage. Furthermore, the coupling between theta and low-gamma oscillations did not show these changes. These results suggest that high-gamma and low-gamma oscillations play different roles in rule switching, and process of trial and error affects the coupling between theta and high-gamma oscillations.

Disclosures: T. Nakazono: None. **S. Takahashi:** None. **Y. Sakurai:** None.

Poster

462. Learning and Memory: Gamma and Theta Rhythms

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant 0090451

Title: Hippocampal theta across its areal axis: predicting, preparing or manipulating future locomotor speed?

Authors: *L. L. LONG[1], I. H. STEVENSON[1,2], M. A. ESCABI[2,3], J. J. CHROBAK[1]; [1]Psychology, [2]Biomed. Engin., [3]Electrical and Computer Engin., Univ. of Connecticut, Storrs, CT

Abstract: The hippocampal theta rhythm is a model system for understanding the temporal organization of neuronal discharge and ensemble communication within and across brain structures. While there have been significant advances in understanding the underlying neurobiology of this rhythm, the relationship of theta to emergent function continues to present a challenge. The present study exposed rodents to environments hypothesized to produce polarized locomotor-theta correlations and we examined dynamic changes in the relationship between theta amplitude and locomotor speed along the longitudinal axis of the hippocampus, as well as the entorhinal cortex. Using cross-correlation analyses between instantaneous theta amplitude and locomotor speed, we demonstrate that during open field navigation septal hippocampal theta

is instantaneously related to locomotor speed. In contrast, during navigation in a linear track, septal and entorhinal cortical theta was highly prospective and preceded changes in overt behavior by hundreds of milliseconds. Further, this time lag relationship systematically decreased across the long axis of the hippocampus. Changing open field task demands, which required linear track-like behavioral output, systematically increased the time lag between speed and theta over days. These results indicate that behavioral output as dictated by prediction of food reward location can strongly modulate speed-theta dynamics allowing the hippocampus and entorhinal cortex to engage in predictive or instantaneous integration of sensorimotor information. The results are interpreted with respect to models of hippocampal function emphasizing neuronal network dynamics that allow for the integration of incoming sensory experience with neural predictions of future sensorimotor experience.

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Poster

462. Learning and Memory: Gamma and Theta Rhythms

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R15 AREA Award 1R15AG045820-01A1

Title: Diffusion-mapped delay coordinates characterize attractor clustering in hippocampal network dynamics during spatial navigation

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Abstract: The hippocampus resides within a cortical neural network that provides a geometric reference frame for spatial navigation. In rodents, hippocampal pyramidal cells code for specific locations in a testing environment, termed place cells. Place cell activity can be decoded to reliably predict animal position and intended path. Therefore, much attention has focused on spiking activity in place cell networks. While the animal is immobile, transient discharge events during sharp-wave ripples (SWRs, 100-250 Hz) code for the most recently taken route or prospectively map the next intended path, termed "trajectory events." During mobile execution of the intended trajectory, place cell spiking precedes the animal's movement, termed "look ahead." Trajectory events and look ahead are coordinated by hippocampal population oscillations

in the slow gamma (25-50 Hz) and theta (4-12 Hz) bands, respectively. These bands are typically analyzed in isolation. However, an understanding of additional relationships between theta, gamma, and SWRs during spatial navigation behaviors may provide greater insight into how patterned discharge events that relate to past or future behaviors emerge. Diffusion mapped delay coordinates (DMDC) is an algorithm for reconstructing dynamical attractors from data using time-delay embedding and diffusion maps. By estimating the most stable component of the dynamics of high dimensional time series data the algorithm can be used to discriminate and quantify distinct dynamical states. It is therefore well suited for identifying dynamics in local field potentials (LFPs). In this work, Long Evans rats were implanted at postnatal day (P) 14 with bipolar stereotrodes terminating in the radiatum of hippocampal area CA1. LFP activity was recorded in synchrony with overhead video at P24 as the animals engaged in spontaneous alternation behavior in a Y-maze. Analysis of LFP data using DMDC revealed distinct dynamical states that were related to specific maze behaviors. Closer investigation of these states allowed characterization of attractor dimension. The dimension quickly converged for epochs corresponding to deliberation at a choice point, while grooming epochs took an additional order of magnitude to converge. On average, a higher dimension attractor was found during center deliberation behavior vs. grooming. Continuing analyses will assess the differential dimensionality of CA1 oscillatory activities during alternation vs. non-alternation choices as well as arm exploration. As spatial navigation ability emerges around P21 in rodents, LFP dynamics just under vs. just over P21 will also be investigated.

Disclosures: **D.G. McHail:** None. **T. Berry:** None. **J.R. Cressman:** None. **T.C. Dumas:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: NEI RO1 EY024056

Title: Memory-guided drawing training increases Granger causal influences from the perirhinal cortex to V1 in the blind

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Abstract: The perirhinal cortex (PRC) is a medial temporal lobe structure that has been implicated in not only visual memory in the sighted, but also tactile memory in the blind

(Cacciamani & Likova, 2016). It has been proposed that, in the blind, the PRC may contribute to modulation of tactile memory responses that emerge in low-level "visual" area V1 as a result of training-induced cortical reorganization (Likova, 2012; 2015). While some studies in the sighted have indicated that the PRC is indeed functionally and structurally connected to the visual cortex (Clavagnier et al., 2004; Peterson et al., 2012), the PRC's direct modulation of V1 is unknown—particularly in those who lack the visual input that typically stimulates this region. In the present study, we tested Likova's PRC modulation hypothesis; specifically, we used fMRI to assess the PRC's Granger causal influence on V1 activation in the blind during a tactile memory task. To do so, we trained 8 congenital and acquired blind participants on a unique memory-guided drawing technique previously shown to result in V1 reorganization towards tactile memory representations (Likova, 2012). The tasks (20s each) included: tactile exploration of raised line drawings of faces and objects, tactile memory retrieval via drawing, and a scribble motor/memory control. FMRI before and after a week of training on these tasks revealed a significant increase in PRC-to-V1 Granger causality from pre- to post-training during the memory drawing task, but not during the motor/memory control. This increase in causal connectivity indicates that the training strengthened the top-down modulation of visual cortex from the PRC. This is the first study to demonstrate enhanced directed functional connectivity from the PRC to the visual cortex in the blind, implicating the PRC as a potential source of the reorganization towards tactile representations that occurs in V1 in the blind brain (Likova, 2012).

Disclosures: L. Cacciamani: None. **L.T. Likova:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

Location: Halls B-H

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Program#/Poster#: 463.02/LLL36

Topic: H.02. Human Cognition and Behavior

Support: NIH R00 AG036845

1UL1TR001430

Title: Aerobic fitness and hippocampal subfield volume in young adults

Authors: *M. F. DUNNE[1], R. K. NAUER[2,3], K. SCHON[1,3,2];
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Abstract: Studies in both humans and rodents highlight a relationship between aerobic fitness, exercise, and hippocampal neuroplasticity. This relationship is postulated to be driven by adult neurogenesis, which occurs in the dentate gyrus (DG) subregion of the hippocampus. Animal studies have shown that aerobic exercise is a potent stimulator of cell proliferation and neurogenesis in the DG. While current limitations of neuroimaging in humans prevent directly measuring the rate of neurogenesis in the living human brain, aerobic fitness and hippocampal volume have been shown to display a positive correlation across the human lifespan. However, there is currently no evidence of an association between aerobic fitness and hippocampal subfield volume in young adults. With the use of high-resolution (0.4 x 0.4 x 2.6mm^3) T2-weighted structural magnetic resonance imaging, hippocampal subfields (subiculum, CA1, and DG/CA3) and medial temporal lobe (MTL) cortices (entorhinal, perirhinal, and parahippocampal) were manually segmented in 20 young adults (mean age 25.6 ± 4.4) of varying aerobic fitness. To determine fitness levels, participants performed a submaximal, graded incremental treadmill test following a modified Balke protocol to estimate VO_2 max, which was then converted to fitness percentile using ACSM norms based on age and gender (median 55th percentile). Hippocampal subfield and MTL cortex volumes were corrected for differences in intracranial volume, which was measured by manually traced mid-sagittal intracranial area. Based on the previous literature, we hypothesized that DG/CA3 and entorhinal cortex would both exhibit a positive correlation with fitness percentile. Preliminary results showed no correlation between entorhinal cortex volume and fitness or total hippocampal volume and fitness in contrast to previous work from our lab and others. However, as predicted at the subfield level we observed a significant positive correlation between DG/CA3 and fitness. Conversely, we found a significant negative correlation between CA1 and fitness. The opposing associations between subfields CA1 and DG/CA3 with fitness explain the lack of a relationship between fitness and total hippocampal volume. Our results extend previous work in humans to the hippocampal subfield level, and are in line with animal models showing that the DG/CA3 region selectively responds to exercise.

Disclosures: **M.F. Dunne:** None. **R.K. Nauer:** None. **K. Schon:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

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Program#/Poster#: 463.03/LLL37

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1539067

Title: The influence of adolescent hippocampal volume and functional connectivity on memory performance: a cross-sectional investigation from the Dev-CoG project

Authors: *D. E. WARREN[1], A. RANGEL[2], J. M. STEPHEN[3], V. D. CALHOUN[3], Y.-P. WANG[4], T. W. WILSON[1];
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Abstract: Hippocampus is known to be necessary for normal declarative memory, but the effects of adolescence on hippocampal volume, function, and related cognitive processing are not well characterized. Although relatively little is known, adolescence is predicted to be associated with significant changes in the volume and/or functional connectivity of hippocampus because of substantial differences in declarative memory performance between children and adults. The Developmental Chronnecto-Genomics project (Dev-CoG) is an ongoing NSF-funded effort to better understand adolescent brain changes and will conduct repeated structural and functional MRI scans of the brains of 230 children (9-15 years of age). Here, we report preliminary findings from the current Dev-CoG dataset, which provide an opportunity to investigate adolescent changes in hippocampus and cognition. Based on neuroimaging and neuropsychological data collected from children and adolescents (N=60, age=9-15) for the Dev-CoG project, we performed a cross-sectional analysis of the developmental course of hippocampal volume and resting-state functional connectivity (rs-FC) as well as behavioral performance on a measure of declarative memory. The test of declarative memory was the NIH Toolbox Picture Sequence Memory Test. Hippocampal volume was measured through manual tracing of the hippocampus from T1 MRI (1×1×1 mm). These hippocampal masks were next used as seed regions for an exploratory whole-brain analysis of hippocampal rs-FC. Resting-state fMRI (rs-fMRI) data were collected using two identical multiband sequences (one eyes-open, one eyes-closed): voxel size, 3.3×3.3×3.0 mm; TR, 460 ms; TE, 29 ms; duration, 306 s (i.e., 650 measurements). Standard functional MRI preprocessing was applied to the rs-fMRI data followed by best-practices processing for rs-FC analyses (Power et al., 2014), including warping of individual data to appropriate atlas space for group analysis. We evaluated age-related changes in these measures of memory performance, hippocampal volume, and seed-based hippocampal rs-FC as well as the interrelated changes in these measures with age. The results provide evidence that adolescent brain development exerts selective effects on memory, hippocampus, and a broader network of brain regions supporting memory and depends on several factors. By evaluating the volumetric, functional, and behavioral consequences of adolescent brain development for hippocampus, this study addresses an important gap in our current understanding of how memory systems develop and further motivates the longitudinal collection of large datasets from child and adolescent populations.

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Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

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Topic: H.02. Human Cognition and Behavior

Support: NIH AG034613

Title: 3T hippocampal metabolites reflect verbal memory decline in aging

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Abstract: The hippocampus experiences a variety of structural and functional alterations that are involved in age-related (and dementia-related) changes in memory. Here, we used short echo time magnetic resonance spectroscopy (MRS) to investigate the age-related changes in metabolite levels in 41 volunteers (20 younger, aged 20-35 and 21 older aged 59-74) and to determine whether metabolite levels correlated with performance on standard neuropsychological tests. ^{1}H spectroscopy data were acquired at the 3T Philips MRI using short echo time PRESS (TR/TE = 2000/37 ms), from a 2.5x1.5x1.0 cm^3 voxel in the left and right hippocampi of all subjects. Voxel dimensions were chosen to maximize the volume largely contained within the hippocampus of each subject. No significant age-related differences were observed bilaterally for Creatine (Cr), N-Acetyl-Asparate (NAA), a combined score for Glutamate and Glutamine (Glx), or Choline (Cho). There were clear age-related declines on cognitive assessments including the Rey-Auditory-Verbal Learning Task (RAVLT) and Trails B. In examining the relationship between metabolites and cognitive performance, an ANCOVA revealed that these two cognitive scores accounted for the variance in metabolite concentrations. Specifically, we found that there was a strong positive correlation between Glx and RAVLT in the whole population. This relationship appeared to be driven by the aged participants, however, as they showed a reliable correlation here ($p=0.06$) while young participants did not ($p=0.6$). A similar pattern, albeit less robust and showing a negative correlation, was found with left hemispheric Cho and Trails B. Furthermore, 14 of the aged adults had previous RAVLT scores from 3-5 years previously (using an alternate version of the task). In this group, we found that right hemispheric Cr and Glx correlated with the difference in RAVLT delayed recall scores over time. Glx was shown to correlate negatively with declining performance, whereas Cr showed evidence of an increase with cognitive decline. These data suggest that MRS measures may be useful in predicting age related verbal memory decline before any physical abnormalities are present.

Disclosures: **S. Nikolova:** None. **S. Stark:** None. **C.E. Stark:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

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Topic: H.02. Human Cognition and Behavior

Support: NIH 5R37AG006265

Title: A longitudinal lifespan study of diffusivity changes in limbic tracts and decline of episodic memory in normal aging

Authors: *Z. SONG, D. C. PARK;
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Abstract: Episodic memory (EM) is particularly vulnerable to aging. White matter tract integrity in the hippocampus-centered memory network is essential to maintain normal episodic memory. Here we investigated in a lifespan sample (aged 20-89, N=199) changes in the fornix and parahippocampal cingulum, two major limbic tracts connected to the hippocampus, and their relationships to EM decline. Diffusion tensor imaging (DTI) and EM performance of cognitively normal adults were studied at two time points (Time 1 & 2) spaced roughly 3.5 years apart in the Dallas Lifespan Brain Study. ROI-based DTI analyses were conducted on the fornix and parahippocampal cingulum based on the ICBM-DTI-81 white matter atlas. The major findings with respect to age-related changes in white matter were as follows: (a) radial diffusivity (RD) of the fornix increased in middle-aged adults from Time 1 to Time 2 (aged 40 to 59, N=62, t=3.20) but not in the young adults (aged 20-39, N=49). The increase was more prominent in older adults (aged 60 to 89, N=88, t=12.23); (b) RD of the parahippocampal cingulum increased in older adults (t=5.47) but not in the middle or young age; (c) axial diffusion (AD) of both tracts increased in older adults (t>3.50) but not in the middle or young age. Most important were the analyses that examined the effect of age and diffusivity changes in these two limbic tracts on EM change, for which global diffusivity change of major white matter tracts in the brain was controlled. Our results indicated that (a) within the elderly group (aged 60 to 89), there was a main effect of RD change of fornix (t=-2.43, p=0.02) and its interaction with age (t=2.00, p=0.05) because that RD change was more strongly related to EM change in the younger elderly compared to the oldest; (b) a similar main effect of AD change of the fornix (t=-2.20, p=0.03) and its marginal interaction with age (t=1.79, p=0.08); (c) for young and middle-aged adults, change in neither RD nor AD of the fornix was related to EM change; (d) change in neither RD nor AD of the parahippocampal cingulum had an effect on EM changes in any of the three age groups. In summary, we found that increases in both RD and AD of the fornix were related to EM decline in cognitively normal elderly, with the effects more pronounced in the younger elderly. Increase in RD of white matter tracts is often related to myelin destruction.

Microstructural mechanisms underlying increases in AD is more complex but possibly related to axonal loss. Therefore our data suggest that degradation of white matter tracts of the fornix in aging may compromise hippocampal connectivity in the core memory network, which then leads to episodic memory decline.

Disclosures: Z. Song: None. **D.C. Park:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

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Topic: H.02. Human Cognition and Behavior

Support: ODNI IARPA Contract 2014- 13121700004

Title: Hippocampal viscoelasticity mediates the benefits of aerobic fitness on memory in healthy young adults

Authors: *H. SCHWARB[1], C. L. JOHNSON[2], A. M. DAUGHERTY[1], C. H. HILLMAN[1], A. F. KRAMER[1], N. J. COHEN[1], A. K. BARBEY[1];
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Abstract: The relationship between cardiovascular fitness and better memory performance is well-established across the lifespan. Better cardiovascular fitness appears to harbor larger hippocampal volumes among developing children and may slow age-related shrinkage to confer better memory ability. Yet, evidence of this relationship among healthy young adults, for whom the hippocampus is neither developing nor atrophying, is less consistent. Volume, however, is a gross measure of structural integrity and the neural underpinnings of memory. In fact, microstrucutral differences in hippocampal integrity may exist even among healthy young adults when volumetric differences are not diagnostic of tissue health or cognitive function. Indeed we have previously reported a strong positive relationship between relational memory performance and hippocampal viscoelasticity, an index of microstructural integrity from magnetic resonance elastography (MRE); however, very little is known about health correlates to this novel measure. In the current study, we investigated (for the first time) the relationship between hippocampal viscoelasticity and cardiovascular health, and their mutual effect on relational memory in a group of healthy young adults (N=51). We replicated our previous finding that hippocampal viscoelasticity correlates with relational memory performance. We extend this work by demonstrating that better cardiorespiratory fitness, as measured by V02 max, was associated with hippocampal viscoelasticity that mediated the benefits of fitness on memory function.

Hippocampal volume, however, did not account for individual differences in memory. Therefore, these data suggest that hippocampal viscoelasticity may provide a more sensitive measure to microstructural tissue organization and its consequences to cognition, even among health young adults.

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Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

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Topic: H.02. Human Cognition and Behavior

Support: MRC Programme Grant G1002276-98624

CHRAT studentship

Title: Functional MRI of language and memory lateralisation for presurgical evaluation of paediatric epilepsy

Authors: *S. M. BUCK[1], T. BALDEWEG[2], D. W. CARMICHAEL[3], R. ELWARD[2], F. VARGHA-KHADEM[2];
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Abstract: Most studies using functional Magnetic Resonance Imaging (fMRI) in paediatric temporal lobe epilepsy (TLE) have focused on language lateralisation and neglected to assess memory. Early onset seizures interfere with the normal process of hemispheric lateralisation and may result in reorganisation of memory and language in paediatric patients. Impairments in memory and learning are frequently reported in TLE patients which is not surprising given that the hippocampus plays a major role in both the generation and spread of temporal lobe seizures and autobiographical and episodic long term memory. Such deficits significantly impact the quality of life and educational progress of the patients. We have developed an fMRI paradigm to assess recall memory, known to be dependent on the integrity of the hippocampus, and language, in children undergoing epilepsy surgery.
Functional MRI correlates of language and memory lateralisation were acquired on a 3T Siemens system in healthy volunteers. Language laterality was assessed through verb generation, where nouns were heard one at a time (one every 4.18 sec) and participants were to overtly

generate a verb for each noun (eg. hear "animal", generate "running"). Memory laterality was assessed through cued recall, where cues were presented to guide recall of previously encoded words (from the language task). Cues consisted of two-phoneme word stems created from the words heard (for example "aen" as a cue for "animal"). The baseline tasks involved a simple counting block and a rest block.
Block-wise comparisons for the language task demonstrate activations in posterior inferior frontal gyrus (Broca's area) and in superior temporal gyrus bilaterally (including Wernicke's area) ($p<0.05$ (FWE)). Similar analyses computed for the memory task demonstrate activations in the temporal lobe, including the hippocampi ($p<0.001$).
Our findings represent predicted brain activations in the language and verbal recall networks, and confirm the feasibility of assessing lateralisation of language and memory within the same session. These brain activations represent interacting networks of language and recall memory. Using this protocol, we evaluate (a) the interdependence of language and memory lateralisation, and (b) the status of those patients who may be at risk of memory impairment after temporal lobectomy.

Disclosures: S.M. Buck: None. **T. Baldeweg:** None. **D.W. Carmichael:** None. **R. Elward:** None. **F. Vargha-Khadem:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 463.08/LLL42

Topic: H.02. Human Cognition and Behavior

Support: NIH AG032361

Title: KIBRA polymorphism and hippocampus-associated integrity in middle age: a multi-modal neuroimaging investigation

Authors: *L. E. KORTHAUER[1], N. T. NOWAK[2], H. SCHERKENBACH[1], E. A. AWE[1], M. FRAHMAND[1], I. DRISCOLL[1];
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Abstract: A single nucleotide polymorphism (SNP) in the *KIBRA* gene (rs17070145) has previously been associated with memory performance and hippocampal volume in older adults, consistent with reports that this SNP is a risk allele for Alzheimer's disease. The purpose of the present investigation was to characterize the relationships between a genetic polymorphism in *KIBRA* and hippocampus-related brain integrity in healthy, middle-aged adults, which may serve

as potential biomarkers of cognitive decline and dementia prior to any overt impairment. Participants (age 40-60, M = 49.9, N = 150) completed the hippocampus-dependent transverse patterning discriminations task (TPDT) and underwent a multi-modal neuroimaging paradigm including T1-weighted structural imaging, diffusion tensor imaging (DTI), magnetic resonance spectroscopy (^{1}H MRS), and resting state functional MRI. Measures of brain integrity included hippocampal volume, white matter (WM) microstructural organization, hippocampal biochemistry, and functional connectivity. Consistent with the literature (Papassatiropoulos et al., 2006), T allele carriers had marginally larger volumes of the right and left hippocampus (p's < .1). In addition, T allele carriers had higher fractional anisotropy (FA), lower mean diffusivity (MD), and lower radial diffusivity (RD), indicating better WM microstructural organization, in the right anterior thalamic radiation, right uncinate fasciculus, and right fornix. These pathways connect the hippocampus with other subcortical and cortical areas. Additionally, better microstructural organization was observed in the genu and body of the corpus callosum and right superior longitudinal fasciculus. T carriers also had higher concentrations of glutamate (Glu) in the left hippocampus (p = .03), consistent with the role of this excitatory neurotransmitter in facilitating episodic memory (Rupsingh et al., 2011). Associations with functional connectivity were not significant. This pattern of better hippocampus-associated structural brain integrity and related WM pathways among T allele carriers was associated with better performance on the TPDT (p's < 0.01). Collectively, these findings suggest a relationship between *KIBRA* polymorphism and hippocampus-associated memory and structural integrity in middle age, decades before the onset of cognitive decline.

Disclosures: L.E. Korthauer: None. **N.T. Nowak:** None. **H. Scherkenbach:** None. **E.A. Awe:** None. **M. Frahmand:** None. **I. Driscoll:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 463.09/LLL43

Topic: H.02. Human Cognition and Behavior

Title: Using eye movements to dissociate memory performance in normal and pathological aging

Authors: *J. K. BLUJUS, C. M. KAIVER, E. I. GRACIAN, K. J. JENNETTE, D. E. HANNULA, I. DRISCOLL;
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Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, particularly in the domains of learning and memory. Normal aging is also associated with cognitive decline, making it difficult to differentiate individuals who will age well from those at risk for developing AD. It is imperative to identify individuals at risk for pathological age-related cognitive impairment prior to the expression of clinical symptoms. Eye movement behavior is a sensitive index of learning and memory. Eye-movement-based relational memory effects emerge rapidly (within 500-750ms of stimuli onset) and precede explicit recognition decisions in healthy, cognitively intact individuals, regardless of task demands. The same effects are disrupted in patient populations. Specifically, eye-movement based relational memory effects are completely absent from the viewing patterns of patients with hippocampal amnesia. Therefore, eye movement measures may have utility in attempts to distinguish normal, age-related decline from pathological cognitive impairment. We tested healthy, community dwelling middle-aged (N=13; age 40-55) and older (N=23, age 65-80)adults and used the Montreal Cognitive Assessment to identify individuals performing in the Mild Cognitive Impairment range ("at risk"), as MCI has been a suggested prodrome of dementia. Participants then completed the Scene-Face Pair Task, comprised of an encoding phase immediately followed by a testing phase. The encoding phase consisted of three study blocks, 42 unique scene-face pairs in each block. Eye movements were recorded during testing while participants viewed 3-face displays superimposed atop previously studied scenes. Participants were asked to indicate whether the matching face (i.e. the associate of the scene) was present or absent. Middle-aged, and older participants directed viewing disproportionately to the matching face within 500-750ms of 3-face display onset whereas greater than chance viewing was not evident in "at risk" participants until 1000-1250ms. All of the participants distinguished target-present from target-absent displays, although recognition performance was decremented among "at risk" individuals. It is possible that this subtle reduction in recognition memory performance is a consequence of response mapping difficulties or general cognitive decline. Further research is needed to identify whether the delay in emergence of eye-movement-based relational memory effects may serve as a useful biomarker to identify individuals succumbing to disease prior to any overt clinical symptoms.

Disclosures: J.K. Blujus: None. **C.M. Kaiver:** None. **E.I. Gracian:** None. **K.J. Jennette:** None. **D.E. Hannula:** None. **I. Driscoll:** None.

Poster

463. Medial Temporal Lobe: Normal and Pathological Memory Through the Lifespan

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 463.10/LLL44

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust

ARUK

BRACE

Title: Healthy ageing and memory consolidation

Authors: *A. WEARN, S. DILLON, H. K. ISOTALUS, D. TSIVOS, M. J. KNIGHT, B. MCCANN, R. A. KAUPPINEN, E. J. COULTHARD;
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Abstract: Healthy humans expect to be able to remember information over days and weeks, or even longer. Episodic memory declines with age and diseases such as Alzheimer's disease (AD), and it is possible that very subtle deficits in long term memory could be the first detectible cognitive problems in AD. Clinically, delayed memory is almost never tested after 30 minutes and there is very little information about rates of forgetting over days and weeks in healthy ageing and neurodegenerative disease.

The hippocampus has a role in stabilisation of memories such that they are retained over long periods – memory consolidation. Pathologically, hippocampus is affected early in AD with CA1 and subiculum among the first subfields to lose volume. The distinct contributions of human hippocampal subfields to memory consolidation are not clear, although, in rodents, it is known that subiculum is critical for sharp wave ripples thought to underpin memory consolidation during sleep. We hypothesised that human long term memory would decline with age and particularly subiculum and CA1 volume would correlate with very long term memory ability in older people.

We tested memory consolidation in 27 healthy elderly people (11 male; mean age 69.3 ± 6.86 SD) using an adapted Hopkins Verbal Learning Test, with Immediate Recall (IR1-3) and delayed recall at 20 minutes, 24 hours, 48 hours, 7 days and 14 days. Participants also performed Montreal Cognitive Assessment (MoCA) and underwent T2-weighted MRI on 3T scanner using an in-house developed CPMG-like sequence (FOV = 184 x 218 x 58mm^3; slice thickness = 1.72mm; in-plane resolution = 0.34 x 0.34 mm^2; TR = 5500; echo spacing = 12ms; 12 echoes). Hippocampal subfields CA1, CA2, CA3, dentate gyrus, subiculum and stratum lacunosum/stratum radiatum/stratum moleculare were segmented on FSL using an in-house developed manual protocol and normalised to total brain volume.

Memory was remarkably well retained over 14 days: 100% retention in 44% of participants at 14 days. Only IR1 performance was significantly lower, as would be expected. Interestingly, memory consolidation did not show any significant correlation with age, estimated IQ or MoCA score. Females consistently scored significantly higher than males, but only reaching significance at IR3 (**P=0.0012). Preliminary analysis suggests a positive correlation between verbal recall at 48h and Left CA2 (**P=0.0013; ρ=0.971; n=6) possibly implicating this region in memory consolidation.

At present we have not confirmed our hypothesis and future work will employ a larger sample size. We also plan a harder consolidation task to reduce ceiling effects and potentially stratify individuals more widely.

Disclosures: **A. Wearn:** None. **S. Dillon:** None. **H.K. Isotalus:** None. **D. Tsivos:** None. **M.J. Knight:** None. **B. McCann:** None. **R.A. Kauppinen:** None. **E.J. Coulthard:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.01/LLL45

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH103421

Title: Does the social cognition enhancing effect of acute oxytocin persist with chronic administration?

Authors: ***D. FEIFEL**, P. D. SHILLING, G. MELENDEZ, J. TRAN, B. ROBERTS, A. AVALOS, W. THUY-UYEN, A. SRIVASTAVA, P. KISHORE, I. DAMANI;
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Abstract: Background: Impaired social cognition is a debilitating feature of several neuropsychiatric disorders and there is a great need for effective treatments. Acute administration of oxytocin improves social cognition raising hope that OT or OT mimetics may be effective treatments for social cognition deficits. However, the extant literature consists primarily of studies that investigated the effects a single OT administration, whereas the chronic effects are more relevant to OT's potential use as a treatment. To investigate the effects of chronic OT on social cognition deficits, we administered daily subcutaneous (SC) OT to Brown Norway rats, an animal model that exhibits natural social cognition deficits. Methods: Male and female Brown Norway rats were administered either SC saline, 0.04, 0.2 or 1.0 mg/kg OT daily for 22 days. On the first and last day of OT treatment each rat's social recognition ability was tested in a social discrimination (SD) task, 40 min after their OT injection. A preference to interact with a novel con-specific over a familiar one is an indicator of intact social recognition in the SD task. In addition, animals were tested 1 day and 7 days after the last OT treatment to evaluate the enduring effects of OT on social recognition. Results: After a single injection, social recognition was exhibited by animals that received any of the OT doses, but not in those that received saline. After 22 daily treatments the magnitude of OT's effect on social recognition was reduced but not eliminated. This reduction was more prominent in males and in animals treated with the high dose. Seven days after OT treatments were stopped, rats treated with OT exhibited greater social recognition than those treated with saline. This persistent OT effect was least apparent in the high dose OT group. Discussion: This study confirms that a single peripheral administration of OT can reverse social recognition deficits. This effect persists after chronic

peripheral OT administration but produces partial tolerance. The persistence of restored social recognition, for at least seven days after cessation of treatment, indicates that chronic OT produces durable neural changes in social circuits. Although the highest dose of OT (1 mg/kg) produced the greatest social recognition improvement in both males and females, lower doses (0.2 mg/kg in males and 0.04 mg/kg in females) exhibited less tolerance and more post-treatment persistence of social recognition. These results support the possibility that OT or OT mimetics may be effective treatment for social cognitive deficits and these results should help inform dose selection in future clinical studies.

Disclosures: **D. Feifel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DF is a named inventor on a patent application for the therapeutic use of oxytocin, filed on his behalf by UCSD. **P.D. Shilling:** None. **G. Melendez:** None. **J. Tran:** None. **B. Roberts:** None. **A. Avalos:** None. **W. Thuy-uyen:** None. **A. Srivastava:** None. **P. Kishore:** None. **I. Damani:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.02/LLL46

Topic: H.02. Human Cognition and Behavior

Title: Facial responses to experienced and observed affective touch

Authors: *L. M. MAYO, I. MORRISON, J. LINDÉ, H. OLAUSSON, M. HEILIG;
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Abstract: Affiliative behaviors such as social touch are believed to elicit rewarding responses that enhance positive social interaction. In humans, one selective feature of social touch is speed of stroking. Stroking to the skin at 3 cm/s robustly activates unmyelinated C tactile (CT) afferents and produces positive subjective ratings of “pleasantness”. Here, we aimed to determine whether social touch also elicits positive affective responses as detected by facial muscle movement. We used facial electromyography (EMG) to assess affective responses to experienced or observed social touch in healthy adults (n=30). In one task, subjects received brush strokes to the arm and palm at 3 cm/s (optimal) or 30cm/s (non-optimal speed). In another task, subjects watched short video clips of touch to the arm, touch to the palm, or non-social touch (i.e. touch to a wooden arm) at 3 and 30 cm/s. After each stimulus, subjects rated how “Pleasant,” “Intense,” and “Relaxing” they found the stimulus. Affective responses to the stimuli were assessed in real-time via EMG recordings of the zygomatic (“smile”) and corrugator

("frown") muscles. Experienced touch at 3 cm/s elicited positive affective responses, including decreased corrugator and marginally increased zygomatic reactivity, while touch at 30 cm/s produced enhanced corrugator reactivity. In addition, participants rated 3 cm/s touch as more pleasant, less intense, and more relaxing. There were no significant differences between touch locations (arm, palm). Observed touch elicited similar patterns of differential affective responding based on velocity, with 30 cm/s videos eliciting increased corrugator reactivity as compared to 3 cm/s videos. Similarly, videos of social touch at 3 cm/s were rated as more pleasant, less intense, and more relaxing than videos of 30 cm/s. However, videos of non-social touch (to the wooden arm) were not rated as pleasant or relaxing at any velocity. We have shown that CT-optimal stroking velocities elicit positive affective responses as assessed by facial EMG. The differential affective responses based on stroking speed were consistent across modalities (felt, seen), consistent with a common coding framework between experienced and observed touch. Observed touch responses were specific to socially-relevant stimuli, indicating that the social nature of the touch rather than the visual features are relevant in eliciting affective facial EMG responses and hedonic ratings. Together, these findings broaden our understanding of affective, socially relevant touch by demonstrating that affective facial responses track stimulus factors related to the social relevance and valence of touch.

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Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.03/DP08 (Dynamic Poster)

Topic: H.02. Human Cognition and Behavior

Support: The Defense Advanced Research Projects Agency contract number W31P4Q12C0166

JUMP Trading Foundation

Title: Intermediate neurodynamic representations: A pathway towards quantitative measurements of teamwork

Authors: *R. STEVENS[1], T. L. GALLOWAY[1], A. WILLEMSEN-DUNLAP[2];
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Abstract: Teams work through coordinated large-scale information exchanges using inter-personal modalities, including speech and action-understandings such as gestures, posture and

other non-verbal communications. It is not surprising that neurophysiologic processes are the underpinnings of many of these interactions and while research is revealing the microscale details of social dynamics, the impact of these studies on team assembly, training, and evaluation has been minimal. One reason is the paucity of quantitative measures of teamwork that adequately span the micro-level social dynamics with the macro-level behavioral dynamics recognized and understood by expert raters. Our research suggests that intermediate representations of team members' EEG-derived variables can be developed that bridge this gap. We describe a symbolic neurophysiological representation that quantitatively portrays the second-by-second neurodynamics of healthcare and submarine navigation teams while they conducted realistic training in natural settings, yet also links to larger-scale behavioral dynamics identified by experts. Second-by-second symbolic representations were created of team member's electroencephalographic (EEG) power across the 1-40 Hz EEG spectrum, and quantitative estimates of the changing dynamics were calculated from the Shannon entropy of the data streams. Significant correlations were seen between the symbol streams entropy levels and ratings of team performance by observers using TeamSTEPPS® (healthcare), or Submarine Team Behavior Toolkit (submarine teams) rubrics. These results suggest that the frequency, magnitude, and / or durations of the teams' neurodynamc fluctuations might reflect performance aspects detected by expert raters. The development of quantitative neurodynamc measures of teamwork provides a first step for building an understandable bridge between the theory and practice of social coordination dynamics, and improving team performance.

Disclosures: R. Stevens: None. **T.L. Galloway:** None. **A. Willemsen-Dunlap:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.04/LLL47

Topic: H.02. Human Cognition and Behavior

Title: Preventive effect of suvorexant on night time falls in patients with cognitive impairment.

Authors: S. YAKOU[1], *T. SHIMAZU[2,3], K. TAKAHASHI[4];
[1]Psychiatry, Saitama Neuropsychiatric Inst., Saitama, Japan; [2]2-33-28, Tokyo, Japan; [3]Neurol., Saitama Neuropsychiatrc Inst., Saitama, Japan; [4]Neurol., Saitama Med. Univ., Saitama, Japan

Abstract: BACKGROUND:
Hypnotic treatment has been associated with increased risk for falls in cognitive impairment patients. Suvorexant, a novel dual orexin receptor antagonist was created as a drug for promoting physiological sleep. Unlike existent sleeping pills, it does not have lenitive and antianxiety

function. These characteristics reduce the side effects like muscular atonia, stagger, dependence on and tolerance for medicines. The authors examined the association between hypnotic treatment and falls in cognitive impairment hospitalized patients, focusing on the suvorexant.
METHODS:
This retrospective case-control study was conducted in an acute geriatric ward in Saitama Neuropsychiatric Institute. Medical records, including demographic, clinical, biochemical, and pharmacological variables, of cognitive impairment patients with falls (N = 32), admitted during a 9-Month period, were reviewed and compared with a control group (N = 63) of patients matched for age and gender and without falls.
RESULTS:
The usage rates of antipsychotics, antidepressants, mood stabilizers, and various nonpsychiatric medications were similar in the two groups, except for suvorexant (higher rates in patients without night time falls). There were no significant differences in the anticholinergic burden values, clinical dementia ratings, and comorbidity burden between the two groups.
CONCLUSIONS:
There was no accident of night time falls in cognitive impairment patients with the use of suvorexant. It suggests that suvorexant may prevent night time falls in cognitive impairment patients. In addition, suvorexant significantly improved patients' sleep experience. Further studies are needed in the future.

Disclosures: **S. Yakou:** None. **T. Shimazu:** None. **K. Takahashi:** None.

Poster

464. Social Behaviors and Pharmacology

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Program#/Poster#: 464.05/LLL48

Topic: H.02. Human Cognition and Behavior

Support: Flanders Fund for Scientific Research (FWO projects 1521313N & G.0401.12)

Branco Weiss fellowship of the Society in Science - ETH Zürich

Title: Exploring the potential of oxytocin for enhancing interpersonal motor resonance upon direct eye gaze: a transcranial magnetic stimulation study

Authors: *J. PRINSEN, S. BRAMS, K. ALAERTS;
Dept. of Rehabil. Sci., KU Leuven, Leuven, Belgium

Abstract: Background. Among different social cues from the environment, the eyes constitute a very salient source for initiating social interaction or communication. Interestingly, previous work from our and other labs demonstrated that direct eye contact between two individuals can readily evoke an increased propensity to 'mirror' other peoples' actions. Particularly, using transcranial magnetic stimulation (TMS), we showed that mirror-motor mapping at the level of the primary motor cortex (M1), also known as "interpersonal motor resonance" (IMR), is significantly increased upon the observation of actions accompanied by direct eye contact, compared to the observation of actions accompanied by averted eye gaze.
Objectives. With the present study, we aimed to investigate the role of eye contact on IMR further, and in particular, explored whether administration of the 'prosocial' neuropeptide oxytocin (OT) can influence eye-contact induced IMR. OT is known to play an important role in promoting prosocial behavior and the perception of socially-relevant stimuli, such as eye gaze. To date however, the link between OT and IMR is less clear.
Method. Twenty neurotypical adult males (18-29y) participated in a double-blind placebo-controlled cross-over design including two sessions, separated by one week. They were randomly assigned to receive a single dose of OT (24 IU) or placebo nasal spray at the first and second session. In each session, TMS was used to measure changes in cortico-motor excitability at the level of M1 while participants observed video stimuli of an actress performing simple hand movements combined with either direct or averted gaze. Additionally, eye tracking was performed to evaluate potential changes in spontaneous viewing behavior of the participants.
Results. Preliminary results replicated previous findings indicating IMR-modulations by the gaze direction of the actor, such that IMR during movement observation was enhanced when combined with direct eye contact. These effects were tentatively more pronounced after administration of OT. Interestingly, participants that failed to display eye contact-induced IMR enhancements at baseline, were shown to significantly increase eye contact-induced IMR after a single-dose of OT.
Conclusions. Our results provide indications that a single-dose of OT can promote motor-mirroring of others' movements upon direct eye contact. OT may thus increase the saliency of social cues originating from the eye regions of others, which in turn may promote the propensity of an individual to automatically 'mirror' the actions and behaviors of surrounding others.

Disclosures: J. Prinsen: None. **S. Brams:** None. **K. Alaerts:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

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Program#/Poster#: 464.06/LLL49

Topic: H.02. Human Cognition and Behavior

Support: Swedish Research Council FYF-2013-687

Title: Hedonic responses to touch from strangers depend on the perceived attractiveness of the caresser

Authors: *G. NOVEMBRE[1], R. ETZI[2], I. MORRISON[1];
[1]Linkoeping Univ., Linkoeping, Sweden; [2]Dept. of Psychology and NeuroMi, Milan Ctr. for Neurosci., Univ. of Milan-Bicocca, Milan, Italy

Abstract: Social touch is attracting increasing attention as a specific category of touch with typical psychophysical properties and neural underpinnings. Previous research has shown that a special type of C fibers, the C tactile (CT) afferents, are involved in detecting gentle, affective tactile input on the skin and conveying the information to the central nervous system, with the posterior insula as one of the crucial cortical targets. Despite building on such bottom-up information flow, the hedonic perception and the physiological consequences of affective touch are probably influenced by various sources of top-down information, which in turn results in adaptive behavioral strategies. For instance, it has been shown that the pleasantness of gentle caresses is affected by the belief about the gender of who is stroking, and various affective cross-sensory stimuli (e.g., pictures, odors) impact the hedonic quality of touch experiences. In the present study we aimed to build on this research by investigating how perception of affective touch is influenced by the attractiveness of hypothetical caressers. Thirty-five young participants (15 women) were brushed on the skin while seeing photos of more attractive and less attractive opposite-gender faces, and imagining those people being the caressers. Along with the Attractiveness factor, the factorial design included other two within-subject factors: Site (forearm, palm), and Velocity (3 cm/s, 30 cm/s). Participants were asked to rate the pleasantness of each stimulation, while electrocardiogram activity (ECG) was measured throughout the experiment. Results of the repeated-measure ANOVA showed that participants preferred touch stimuli delivered by more attractive people; a preference for the palm and for the slow velocity (3 cm/s) was also found. The degree to which slow stroking is preferred over fast critically depends on the attractiveness of the face stimuli, reflected by an interaction between Attractiveness and Velocity, with a higher difference for more attractive compared to less attractive. Furthermore, ECG data analysis showed that touch stimuli associated with more attractive faces resulted in significantly higher heart rate variability (HRV) than ones associated with less attractive faces. Overall, the present study confirms that contextual social information plays a major role in affective touch experiences, impacting not only the perceived hedonic quality of the experience but also the physiological state of the body.

Disclosures: **G. Novembre:** None. **R. Etzi:** None. **I. Morrison:** None.

Poster

464. Social Behaviors and Pharmacology

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Senior Investigator Award

Title: Associative learning of what is yours and mine

Authors: *P. L. LOCKWOOD, M. WITTMANN, M. APPS, G. HUMPHREYS, M. RUSHWORTH;
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Abstract: How do we learn what items in the world are "mine" and how do we learn what belongs to other people? Much of our social lives require us to know what objects belong to whom. Previous studies have suggested that people have a 'self bias' in perceptual processing and memory such that they are faster to process stimuli that have been associated with themselves and they are better at remembering information related to themselves. In parallel, studies of decision-making have shown that associative learning occurs by solving a credit assignment problem where credit for an outcome is assigned to a cue rather than an alternative when a prediction is confirmed. However, the computational mechanisms that underpin how self-associations are formed, and the origins of the 'self bias', remain unknown.
Participants performed an associative learning task in which they were required to learn which stimuli belonged to themselves, their best friend or to a stranger. Behaviourally, we find that participants have faster reaction times when associating stimuli with themselves, and correctly identify which stimuli are theirs at a significantly higher proportion compared to their friend or a stranger. Using a computational model of associative learning, we find that participants have a higher learning rate when learning to associate stimuli with themselves compared to a friend or stranger. We also find that participants learn quicker from correct compared to incorrect information across conditions, supporting a credit assignment account of associative learning, but still learn faster after correctly assigning a stimulus to themselves than to a friend or stranger. The neural bases of these effects are yet to be elucidated but the ventromedial prefrontal cortex and superior temporal sulcus have previously been shown to correlate with a self-bias effect. Do these regions encode differences in the associative value of stimuli that are ours and others? and/or do they signal an 'ownership' prediction error that drives learning when feedback is unexpected? On-going work will examine these questions.

Disclosures: P.L. Lockwood: None. **M. Wittmann:** None. **M. Apps:** None. **G. Humphreys:** None. **M. Rushworth:** None.

Poster

464. Social Behaviors and Pharmacology

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Program#/Poster#: 464.08/LLL51

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant 31530032

Title: Oxytocin biases men but not women to restore social connections with individuals who socially exclude them

Authors: *X. XU, S. YAO, L. XU, Y. GENG, W. ZHAO, X. MA, J. KOU, R. LUO, K. KENDRICK;
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Abstract: We normally react to individuals who socially exclude us by either avoiding them or increasing our attempts to interact with them[1]. The neuropeptide oxytocin (OXT) plays a critical role in promoting social bonds and prosocial behaviors and can also reduce social conflict[2]. In the current study we therefore investigated if it facilitates more positive social responses towards individuals who exclude or include us.

In a randomized, double-blind, placebo-controlled, between-subject design 77 healthy Chinese participants (40 males) received intranasal OXT (40 IU) or placebo (PLC) before playing a modified virtual ball-tossing game (Cyberball) with three fictitious partners who either showed exclusion, inclusion or neutral behavioral interactions with them. The Cyberball game was played for~5 min and consisted of six rounds with a total of 60 throws in each one. One week after the initial Cyberball experiment subjects returned to complete a further three tasks but with no additional treatment: a surprise memory test for recognition of previous vs new players, likeability ratings for their face pictures and whether they wanted to play the game again with them.

Results showed that subjects threw the ball more often to individuals who excluded rather than included them (p<.001) or were neutral (p=.001), although OXT did not alter this or awareness/feelings of exclusion or inclusion (p>0.5 in all cases). However, when subjects returned a week later males, but not females, in the OXT group showed increased liking for, and preference for playing again with, players who previously excluded them (Likeability: OXT male: excluder=5.88±0.22, includer=5.22±0.26, p=0.001; OXT female: excluder=5.4±0.23, includer=5.46±0.27, p=0.744; % play again: Male: PLC: 31.67±3.11, OXT: 44.86±2.88, t=-3.12, p=0.003; Female: PLC:
35.65±5.5, OXT: 40.2±4.62, t=-0.64, p=0.53). In addition, this OXT effect in males was positively associated with independent orientation scores (r = 0.585, p = 0.007) on the Individualism and Collectivism scale (ICS). OXT had no significant effect on subsequent

recognition memory for includers or excluders (p>0.07).
Overall our results suggest that when males from a collectivist culture play simultaneously with individuals who socially include or exclude them, OXT treatment increases their motivation to re-establish connections with those who previously excluded them, particularly if they have a higher independent orientation.

References

1. Maner JK et al. (2007) *J. Pers. Soc. Psychol.* **92,** 42.
2. Striepens N et al. (2011) *Front. Neuroendocrinol*. **32,** 426.

Disclosures: **X. Xu:** None. **S. Yao:** None. **L. Xu:** None. **Y. Geng:** None. **W. Zhao:** None. **X. Ma:** None. **J. Kou:** None. **R. Luo:** None. **K. Kendrick:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.09/LLL52

Topic: H.02. Human Cognition and Behavior

Support: FCT SFRH/BD/52201/2013

Title: Is it me moving this? Embodiment over the virtual body is improved by active control

Authors: *V. BRUGADA-RAMENTOL[1], I. CLEMENS[2], Á. ROMÁN[2], G. G DE POLAVIEJA[2];
[1]Fundaçao Champalimaud, Lisbon, Portugal; [2]Champalimaud Res., Lisbon, Portugal

Abstract: The malleability of self-representation to introduce a foreign limb has been a widely studied process in the recent years in the area of cognitive neuroscience. It has been shown that both sense of ownership and sense of agency contribute to the modulation of embodiment over a body. In several studies, the presence of distortions in the foreign limb affected the reported sense of ownership and agency. However, few studies include active control over the fake limb's movement, which is crucial to understand sense of agency. We propose that under active control over a virtual body, sense of agency and sense of ownership should be more resistant to the addition of a discontinuity on the limb. On the other hand, affecting sense of agency through addition of noise to the movement of the virtual hand, should decrease both sense of ownership and sense of agency. In a custom made virtual environment, which allowed the participants to have full control over the virtual arm during a reaching-like task, we found no significant differences in reported sense of ownership and agency when a discontinuity is added to the limb. On another study, we show that the addition of noise in the movement causes a significant

decrease on the reported sense of ownership and agency over the virtual hand. When put together, these results suggest that sense of ownership is strongly affected by sense of agency, and that the modulation of one of these components influences the other. Unexpectedly, in the context of modulating sense of agency, when the hand is threatened, participants do not show a difference in reaction between control and noise conditions. This suggests that implicit and explicit measures of ownership could depend on different cognitive processes.

Disclosures: V. Brugada-Ramentol: None. **I. Clemens:** None. **Á. Román:** None. **G. G de Polavieja:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.10/LLL53

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant 31530032

Title: Oxytocin increases tolerance of infidelity in males but decreases it in females

Authors: *L. XU, R. LUO, X. ZHENG, X. XU, Z. GAO, K. KENDRICK;
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Abstract: Previous studies have suggested that while oxytocin (OXT) may enhance fidelity towards partners in male monkeys[1] and humans[2] it can also decrease arousal in response to descriptions of partner infidelity[3]. Here, in a randomized, double-blind, between-subject, placebo (PLC) controlled design experiment, 160 participants (80 males, aged 18-27 years) received either intranasal administration of OXT (40 IU; n=80) or PLC. Forty-five minutes after intranasal treatment, participants completed a rating task. In this task, participants viewed neutral faces of 40 strangers of the opposite sex paired with sentences describing a behavior indicative of fidelity or infidelity (either emotional or sexual) he/she performed during a past relationship, and then rated their attractiveness, likability and trustworthiness and whether they would like to have a short or long-term relationship with them. The subjects were then given a surprise recognition memory test for the 40 faces and another 40 novel ones. All faces and sentences were selected and counterbalanced based on a pilot rating by 36 subjects (17 males). Results showed significant treatment*gender*fidelity interactions for attractiveness ($p<.01$) and likeability ($p<.05$). In male subjects OXT increased the attractiveness ($p<.05$) and likability ($p<.05$) of members of the opposite sex who had been unfaithful (both sexually and emotionally) in a relationship but in females their attractiveness was decreased ($p<0.05$). No significant OXT

effect was found for trustworthiness and relationship ratings. OXT also made women, but not men, less likely to remember the individuals showing infidelity (treatment*gender*fidelity interactions: $p<0.05$). Thus overall in men OXT increases the attractiveness and likability of female strangers who have previously shown emotional and sexual infidelity whereas in women it makes unfaithful male strangers both less attractive and memorable.

Reference
1.Cavanaugh J et al. (2014). Psychoneuroendocrinology, 49, 1.
2.Scheele D et al. (2012) J Neurosci, 32, 16074.
3.Preckel K et al. (2015) Soc Cognit Affect Neurosci, 10, 987.

Disclosures: **L. Xu:** None. **R. Luo:** None. **X. Zheng:** None. **X. Xu:** None. **Z. Gao:** None. **K. Kendrick:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.11/LLL54

Topic: H.02. Human Cognition and Behavior

Title: Sensory and metacognitive processing is modulated by the mere inferred presence of another individual

Authors: *S. EREIRA[1], Z. KURTH-NELSON[1], S. FLEMING[2], R. DOLAN[1];
[1]Max Planck UCL Ctr., [2]Wellcome Trust Ctr. for Neuroimaing, Univ. Col. London, London, United Kingdom

Abstract: Social context is known to exert measurable effects on cognition and recent advances in behavioural economics and computational imaging have enabled the characterisation of specific examples of social cognition, such as conformity and collective decision-making. However, it has been challenging to isolate specific components of social cognition within an ecologically valid environment, an essential task for developing models of social cognitive deficits, such as in autism and schizophrenia. We developed a novel behavioural paradigm to address whether the mere presence of an other is sufficient to modulate sensory perception. Trios of participants played a perceptual decision making task concurrently on separate computers and simultaneously reported their confidence. On some trials, only one participant engaged in the task, and the others 'sat out'. On other trials, two or three participants independently engaged with the same stimuli. The number of participants engaged was cued to all participants on each trial. However, crucially, participants never saw each other's responses or outcomes. We found that, on trials with more than one participant engaged, the proportion of correct responses was

higher, and confidence was more correlated with difficulty. We propose that social context modulates sensory gain, leading to more accurate representations of perceptual inputs.

Disclosures: **S. Ereira:** None. **Z. Kurth-Nelson:** None. **S. Fleming:** None. **R. Dolan:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.12/LLL55

Topic: H.02. Human Cognition and Behavior

Support: MH080838

Title: How social coordination emerges and changes among multiple heterogeneous agents: An experimental 'human firefly' study

Authors: ***M. ZHANG**[1], J. A. S. KELSO[1,2], E. TOGNOLI[1];
[1]Ctr. for Complex Systems & Brain Sci., Florida Atlantic Univ., Boca Raton, FL; [2]Intelligent Syst. Res. Ctr., Ulster Univ., Derry~Londonderry, Ireland

Abstract: People's behavior, emotion, mental and physical health reflect the social milieu in which they are embedded. Social structures are essentially dynamic: their evolving characteristics depend on the coordinative stability between people's behavior. Dyadic social coordination has been well studied experimentally and mathematically at both component and collective levels. It uncovered metastability as an informationally-rich mix of integration and segregation that depends on coupling strength and differences between people's intrinsic behavior. Whereas the science of coordination (social, neural or physical) tends to polarize toward either systems of 2 or 3 components or systems composed of very many degrees of freedom, much of reality may lie in between. In an attempt to uncover laws and mechanisms of social coordination at this intermediate scale, we introduce a paradigm involving the coordination of rhythmic movement among 8 people. Subjects were seated in obscurity at booths around an octagonal table. Each subject signaled his/her tapping behavior to the group with a touchpad and watched others' taps via a ring of 8 LEDs. Each LED lit up when its assigned individual contacted the touchpad. Subjects were instructed to tap rhythmically to a visual metronome shown at the beginning of each trial (10 s) and to maintain that frequency throughout the following 50s, when they saw each other's tapping via the flashing LEDs. The pacing metronomes were parametrically manipulated to divide the subjects into 2 groups of 4 participants, with frequency differences $\delta f = 0$, 0.3 or 0.6Hz. We investigated whether subjects' behavior would persistently follow their initial group or if they would shift to the behavior of the

other group. Preliminary data show that subjects tended to persist within their initial frequency groups, yet cross-group switching also occurred. The analyses of phase-locking stability reveal that as the frequency difference δf decreases, the correlation between cross-group and within-group phase-locking changes gradually from negative to positive - a zero-correlation marked the critical frequency difference δf^* where original groups begin to merge into one. Our findings suggest that relevant variables to metastable coordination dynamics in dyadic situations still plays an important role in 8 people coordination, but multiagent coordination gives rise to social complexity with formation of groups at multiple scales. The present work opens a new behavioral paradigm for identifying neuromarkers of multi-agent coordination dynamics that may be relevant to clinical populations where social stability and instability are at stake.

Disclosures: **M. Zhang:** None. **J.A.S. Kelso:** None. **E. Tognoli:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.13/LLL56

Topic: H.02. Human Cognition and Behavior

Support: NSFC 31530032

Title: Oxytocin transiently and selectively facilitates acceptance of social advice from others but without increasing their trustworthiness

Authors: *R. LUO, L. XU, W. ZHAO, X. MA, X. XU, J. KOU, Z. GAO, B. BECKER, K. KENDRICK;
Sch. of Life Sci. and Technol., UESTC, Sichuan, China

Abstract: Research using economic game paradigms has suggested that the neuropeptide oxytocin (OXT) can increase willingness to trust others, although these findings have become increasingly controversial and may depend on the specific context. Here we investigated whether intranasal OXT influences trust in advice given on solving everyday social problems by either qualified or unqualified advisors. In a randomized, double-blind, between-subject, placebo-control design study 83 subjects (42 males) received either intranasal OXT (24IU) or placebo (PLC) treatment. On the first study day subjects provided written solutions to 60 everyday social problems. On the subsequent day they self-administered intranasal OXT or PLC. After 45 min they were given the 60 problems again, reminded of their answers and received alternative advice from 4 older advisors who were introduced as either experts (male/female psychological counselors) or non-experts (male/female landscape designers). In each case subjects were shown

a face picture and name of the advisor and were given either better or worse advice by them (randomly computer generated based on 4 top answers and ratings provided by independent groups of subjects) which they could either accept or reject. Subjects returned again one week later and answered the same 60 questions without being reminded of their previous or advised answers. Results revealed no main effects of advisor type or treatment (all p>0.39) but a treatment x advisor type x advisor gender interaction (p=0.026, η^2_p =0.06). OXT selectively increased acceptance of advice from a female (p=0.034, Cohen's d=0.49), but not from a male expert (p=0.792, Cohen's d=5.72) or the male or female non-experts (both p>0.32). Further analysis revealed a main effect of advice-type (p<0.001, η^2_p =0.49), with subjects being less accepting of worse advice from any advisor, but no interactions involving treatment x advice type, indicating OXT increased acceptance of both better and worse advice from the female Psychologist. Importantly, there was no OXT effect on ratings of advisor trustworthiness or likability (all advisors p>0.25). Furthermore, the OXT effect on advice acceptance was not maintained after a week, with subjects mainly reverting to their original solutions irrespective of previous accepted advice being better or worse (all p>0.14). These findings suggest that while OXT can increase trust behavior towards others it does so in an implicit, transient and highly selective person- and gender-specific manner. Thus OXT may have a rather limited utility in terms of making us generally more trusting of others.

Disclosures: **R. Luo:** None. **L. Xu:** None. **W. Zhao:** None. **X. Ma:** None. **X. Xu:** None. **J. Kou:** None. **Z. Gao:** None. **B. Becker:** None. **K. Kendrick:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.14/LLL57

Topic: H.02. Human Cognition and Behavior

Support: NSFC 31530032

Title: oxytocin facilitates emotional empathy for individuals expressing negative emotions in males and females

Authors: *Y. GENG, W. ZHAO, F. ZHOU, B. BECKER, K. KENDRICK;
Sch. of Life Sci. and Technol., UESTC, Sichuan, China

Abstract: Intranasal administration of the neuropeptide oxytocin (OXT) can enhance empathy, although whether both cognitive and emotional empathy are affected and the importance of valence is unclear. We have investigated OXT effects on cognitive and emotional empathy

evoked by viewing individuals expressing positive and negative emotions in two independent studies.
In two randomized, double-blind, placebo-controlled, between-subject design experiments (Exp 1: 60 males, OXT=28; Exp 2: 40 males, OXT=19 and 31 females, OXT =18) we investigated the effects of intranasal OXT at different doses (Exp 1: 24IU; Exp 2: 40IU) vs. placebo (PLC) treatment on cognitive and emotional empathy which were measured using an Asian version of the Multifaceted Empathy Test (MET) used in a previous study on Caucasian subjects. Starting 45 mins after the intranasal spray subjects were given the MET where they were asked to view natural scenes showing individuals expressing strong positive or negative emotions. Each of the 60 scenes was shown three times and subjects required to identify the emotion being expressed from 4 different options (cognitive empathy - CE), or to rate the strength of their feeling for the subject in the scene (direct emotional empathy - EE) or how aroused they were by the picture itself (indirect EE - EEI).
Results from ANOVA analyses showed that in both experiments there was a treatment *X valence interaction in Exp 1 (p=0.032, η^2_p =0.05) and a marginal one in Exp 2 (p=0.058, η^2_p =0.08). Post-hoc tests showed that this was due to OXT having a significant effect only for negative valence stimuli (Exp 1: p=0.025, Cohen's d=0.59; Exp 2 p=0.049, Cohen's d=0.48). Exploratory t-tests revealed that for negative valence stimuli OXT increased EE (p=0.029, Cohen's d=0.58) and EEI (p=0.037, Cohen's d=0.55) but not CE (p=0.937, Cohen's d=0.02) ratings in Exp. 1 and EE (p=0.043, Cohen's d=0.49) but not EEI (p=0.093, Cohen's d=0.40) or CE (p=0.498, Cohen's d=0.16) in Exp 2. There were no sex differences in OXT effects in Exp 2. There was also no evidence for dose-dependent OXT-effects in male subjects in the two experiments.
In summary, our results show that in both male and female Chinese subjects OXT only enhances emotional empathy for negative valence stimuli in the MET, whereas a previous experiment in male Caucasian subjects reported increases in both positive and negative valence using the same paradigm. In agreement with this latter study no OXT-effects on cognitive empathy were found.

Disclosures: **Y. Geng:** None. **W. Zhao:** None. **F. Zhou:** None. **B. Becker:** None. **K. Kendrick:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.15/LLL58

Topic: H.02. Human Cognition and Behavior

Support: FEBS Long-Term Fellowship

Juan de la Cierva Fellowship

Title: Behavioral and molecular individuality in zebrafish is controlled by a YY1/HDAC1/p300 pathway

Authors: *A. C. ROMAN[1], J. VICENTE-PAGE[2], G. GARCIA DE POLAVIEJA[2];
[1]Champalimaud Neurosci. Programme/ Collective Behaviour Lab., [2]Fundaçao Champalimaud, Lisbon, Portugal

Abstract: Behavioral individuality arises even in isogenic populations under identical environments, but its underlying mechanisms remain elusive. We found that inbred and isogenic zebrafish (*Danio rerio*) larvae showed behavioral individuality swimming freely in identical wells or in reaction to stimuli. This individuality was found to be encoded in individual histone-4 acetylation profiles of several genomic regions related to neurodevelopment. The interaction of a complex formed by YY1, HDAC1, and p300 with these regions is required for individuality in acetylation. Manipulations of this complex or its regulators, like acetyl-CoA, simultaneously modified epigenetic, transcriptional and behavioral individuality. These results and computational simulations suggest stochastic histone acetylation by the YY1/HDAC1/p300 complex in neurodevelopmental genes as primary mechanism of molecular and behavioral individuality in vertebrates. This mechanism seems to be partially conserved in other species like humans.

Disclosures: A.C. Roman: None. **J. Vicente-Page:** None. **G. Garcia de Polavieja:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.16/LLL59

Topic: H.02. Human Cognition and Behavior

Title: Using development in zebrafish larvae to extract the rules of collective behavior

Authors: *R. C. HINZ, G. DE POLAVIEJA;
Collective Behavior Lab., Champalimaud Fndn., Lisbon, Portugal

Abstract: Advances in neurobiology and genetics have opened up the possibility to study the cognitive mechanisms and genetic basis of social behavior. However, while there exists a variety of state-of-the-art techniques for brain imaging and manipulation for zebrafish, *Danio rerio*, tools for the quantification and analysis of social behavior in free-swimming animals are scarce. Moreover, strong shoaling behavior can be found in adult zebrafish, while imaging and

manipulation techniques are mostly applied to zebrafish at early stages of development when animals are transparent but show little social interactions.To overcome these difficulties, we have developed idSocial, a toolbox for the analysis of social behavior in freely moving animals. Performing experiments and tracking the interactions between animals in groups of free swimming zebrafish larvae, we have generated a dataset of trajectory data for the first 24 days post fertilization. The analysis with idSocial finds a continuous decrease of inter-individual distances and a continuous increase in attraction from 7-8 dpf on, while the tendency to align the direction of movement stays almost constant during the observed development. At early stages, individuals show strong repulsive interactions with other animals around them and attractive interactions at larger distances are less frequent. During development, the repulsion region shrinks and animals spend an increasing amount of time in attractive interactions.
We found that the properties of attraction are explained, without the need to fit parameters, by a model in which each animal follows another animal chosen effectively at random. The experimental data eliminates models that use more sophisticated rules, and explains collective behavior simply by animals statistically choosing a larger group by randomly selecting an individual. We propose this rule as a basic reference in collective animal behavior. The emergence of collective behavior as an accumulation of different properties during development is expected to help dissecting how different properties of brain processing underlie animal interaction rules.

Disclosures: **R.C. Hinz:** None. **G. De Polavieja:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.17/LLL60

Topic: H.02. Human Cognition and Behavior

Support: JAE PreDoc - PhD Fellowship

Title: Optimal group size in collective decision making

Authors: ***J. VICENTE-PAGE**[1], C. IOANNOU[2], A. PEREZ-ESCUDERO[3], G. DE POLAVIEJA[1];
[1]Champalimaud Res., Fundaçao Champalimaud, Lisboa, Portugal; [2]Sch. of Biol. Sciences, Univ. of Bristol, Bristol, United Kingdom; [3]MIT, Boston, MA

Abstract: Collective decision making has been observed in different animal species, from ants to humans, and the usual modeling theory has always predicted that the accuracy of the decision

increases with group size. This prediction has usually been obtained by the implementation of the models themselves, where individuals are only allowed to vote once and sequentially. This reduces extremely the information of the first deciding individuals, as they never have access to the actions of the rest of the group. Here we present a completely general collective decision making model in which individuals are allowed to reevaluate their decisions in several voting rounds, favoring the information exchange betwen the members of the group. Our model predicts that the optimal accuracy is achieved at intermediate group sizes, as the advantage of having more information in large groups can be penalyzed by the possibility of amplifying initial mistakes. We further confirmed the existence of an optimal group size in collective decisions by conducting a set of experiments in groups of guppies.

Disclosures: **J. Vicente-Page:** None. **C. Ioannou:** None. **A. Perez-Escudero:** None. **G. de Polavieja:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.18/LLL61

Topic: F.02. Behavioral Neuroendocrinology

Title: Early life social isolation in larval zebrafish alters behavior in social and non-social contexts

Authors: *A. H. GRONEBERG[1], J. C. MARQUES[2], M. B. ORGER[2], G. G. DE POLAVIEJA[1];
[1]Collective Behavior Lab., [2]Vision to Action Lab., Champalimaud Res., Lisbon, Portugal

Abstract: Early social experience can have long lasting effects on social behavior and development. Zebrafish are a shoaling species with oviparous development and transparent bodies in the larval stage, allowing for non-invasive imaging of neural activity as well as very controlled manipulation of the environment during very early development. Here we set up to test how the social environment during early development affects the development of social and non-social behavior. We reared wild-type zebrafish in isolation or in groups until 6 days post fertilization and subsequently measured spontaneous locomotion at high temporal resolution, allowing for the analysis of single tail beats. Our results show that when swimming in groups or pairs, isolatedly raised larvae avoid each other at a larger distance than group raised larvae. This difference was absent when the same test was performed in darkness, suggesting that this increased avoidance is visually mediated. However, raising larvae in isolation while allowing visual access to age-matched conspecifics did not rescue the enhanced avoidance reaction.

Further, we noticed several differences in morphology, as well as general locomotion kinetic parameters irrespective of the social context during testing when comparing isolated and group raised larvae. Such differences could not be fully explained by a mere shift or retardation in development. We hypothesized that the absence of non-self sensory stimuli could lead to reduced swimming activity during development and thus affect development of the muscular system. Yet, repeated artificial mechanosensory stimulation failed to rescue the morphological and locomotion phenotypes. In summary, we here characterized a model of social isolation during early development in larval zebrafish. Further molecular, behavioral and neural circuit experiments will allow us to study how the developing brain is altered when exposed to social deprivation early in life.

Disclosures: **A.H. Groneberg:** None. **J.C. Marques:** None. **M.B. Orger:** None. **G.G. de Polavieja:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.19/DP09 (Dynamic Poster)

Topic: H.02. Human Cognition and Behavior

Support: Swiss National Science Foundation

Heffter Research Institute

Swiss Neuromatrix Foundation

Title: The role of the serotonin 2A receptor system in self and other initiated social interaction in LSD-induced states

Authors: *K. H. PRELLER[1], L. SCHILBACH[2], T. POKORNY[1], J. FLEMMING[1], R. KRAEHENMANN[1], P. STÄMPFLI[3], M. LIECHTI[4], E. SEIFRITZ[1], F. X. VOLLENWEIDER[1]; [1]Dept. of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric Univ. Hosp. Zürich, Zurich, Switzerland; [2]Independent Max Planck Res. Group for Social Neurosci., Max Planck Inst. of Psychiatry, Munich, Germany; [3]MR Ctr. of the Psychiatric Univ. Hosp. and the Dept. of Child and Adolescent Psychia, Univ. of Zurich, Zurich, Switzerland; [4]Dept. of Biomedicine and Dept. of Clin. Res., Univ. Hosp. Basel, Basel, Switzerland

Abstract: Distortions of self-representation are critical symptoms of major psychiatric disorders such as schizophrenia. Furthermore, a coherent sense of self is vital for successful participation in social interactions. In light of the immense need for improved treatment of transdiagnostic

social impairment in psychiatric disorders, it is important to better understand the neurochemical substrates of social interaction abilities. In the current study we, therefore, investigated the pharmacological and neural correlates of gaze-based social interaction and joint attention which has been shown to rely upon recruitment of the brain's reward system. In a double-blind, randomized, counterbalanced, cross-over study 24 healthy participants received either 1) placebo+placebo (Pla condition) 2) placebo + lysergic acid diethylamide (LSD) (100 µg p.o., LSD condition), or 3) ketanserin (40 mg p.o.) + LSD (100 µg p.o., Ket+LSD condition) at three different occasions. Participants completed an interactive social paradigm in which their gaze was recorded by an eye-tracking device that controlled the gaze of an anthropomorphic virtual character while undergoing functional magnetic resonance imaging. They completed trials of self- and other-initiated joint and non-joint attention. Activation of dorsal medial frontal cortex and the posterior cingulate cortex observed in a self vs. other contrast was reduced after LSD administration compared to Pla and Ket+LSD.conditions (all $p<0.05$, FWE corrected). Comparing joint attention vs. non-joint attention was associated with reduced BOLD signal in the ventral striatum in the LSD condition compared to Pla. Furthermore, LSD increased ratings on the 5D-ASC scales "experience of unity" and "disembodiment". All LSD-induced effects were blocked by the serotonin 2A receptor antagonist ketanserin (all $p<0.05$). Our study demonstrates that LSD induced a feeling of loosening of self-boundaries, which is consistent with previous reports. Concordantly, LSD reduced neural activity in brain areas, which are known to be important for self-processing, but also social cognition. Reduction of brain activity in reward-related areas during social interaction after LSD administration might further explain social withdrawal symptoms in patients suffering from distortions of self-experience such as schizophrenia. These LSD-induced effects appear to be attributable to serotonin 2A receptor stimulation, since these alterations were normalized after pretreatment with ketanserin.

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Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.20/LLL62

Topic: H.02. Human Cognition and Behavior

Title: Negative gender-related information reduces social cognition in breast cancer patients

Authors: *A. N. SOKOLOV[1], M. A. PAVLOVA[3], S. Y. BRUCKER[1], D. WALLWIENER[2], E. SIMOES[1];
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Abstract: Implicit negative information perceived as a threat impedes visual social cognition (e.g., Pavlova et al., 2014): telling that men are usually better than women on the event arrangement, EA, task (with no initial gender differences) drastically reduces women's performance. When diagnosed with breast cancer, women face with a lot of threatening information that may hinder their cognition, decision making and eventually, coping with the disease. We examined whether gender-related information affected performance on visual social cognition task in patients with mastocarcinoma. Two separate groups of patients (aged 40-55 years) and two control groups of matched healthy women were administered the EA task with standard instruction. In addition, one patient and one control group were told that men were commonly better on the task. With negative information, patients scored lower than controls, and lower than patients with standard instruction, indicating effects of both disease and information. Remarkably, the lowest scores occurred in patients with negative information. The outcome shows for the first time the impact of disease and information on visual social cognition, presumably blocking visual cognitive processing. This offers novel insights on improving physician-patient communication for enhanced visual cognitive processing in oncologic and other diseases.

Disclosures: A.N. Sokolov: None. **M.A. Pavlova:** None. **S.Y. Brucker:** None. **D. Wallwiener:** None. **E. Simoes:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 464.21/LLL63

Topic: H.02. Human Cognition and Behavior

Title: Long-term treatment with methylphenidate for mental fatigue and pain after traumatic brain injury

Authors: *L. 0. RONNBACK[1], B. JOHANSSON[2];
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Abstract: Objectives: Traumatic brain injury (TBI) may cause long-lasting post-concussive symptoms, such as mental fatigue and concentration difficulties and this may become the main hindrance for returning to work, studies and normal social life. There is currently no effective treatment for long-lasting mental fatigue. In this hypothesis generating study the long-term effects of methylphenidate on mental fatigue, cognitive function, pain and safety were assessed. Materials & methods: Thirty participants who suffered from long-term post-concussion symptoms after mild or moderate TBI and who had reported positive effects with methylphenidate during an initial phase of this follow-up study were treated with methylphenidate for a further six months. Results: After six months follow-up, effects on Mental Fatigue Scale (MFS), depression, anxiety and cognitive function (processing speed, attention, working memory) were significantly improved compared to baseline data ($p<0.001$ respectively). Pain was not improved as measured with VAS but the patients tolerated their pain much better. Heart rate was significantly increased ($p=0.01$), while blood pressure was not changed. Conclusions: Individuals suffering from prolonged symptoms after TBI reported reduced mental fatigue and improved cognitive functions with long-term methylphenidate treatment. They tolerated pain better. It is suggested that methylphenidate can be a treatment option for long-term mental fatigue and cognitive impairment after TBI, but further randomized control research is warranted.

Disclosures: L.0. Ronnback: None. **B. Johansson:** None.

Poster

464. Social Behaviors and Pharmacology

Location: Halls B-H

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Program#/Poster#: 464.22/LLL64

Topic: H.02. Human Cognition and Behavior

Support: The authors would like to acknowledge the use of the Advanced Research Computing (ARC) in carrying out this work.

Title: Can everyone see the target? Cross-cultural differences in bottom-up and top-down attention: A computational modelling study.

Authors: *E. MAVRITSAKI[1], P. RENTZELAS[2];
[1]Birmingham City Univ., Birmingham, United Kingdom; [2]Dept. of Psychology, Birmingham City Univ., Birmingham, UK, United Kingdom

Abstract: Can we all see salient items presented to us? Members of collectivist East Asian cultures when compared with individualist European American cultures into picture perceptions

showed that East Asians are more likely to attend to perceptual field as whole and to perceive relationships between a salient object and background than European Americans [1]. The effect was observed when target object presented amongst clearly separated background objects (distractors) and the subject did not look for a specific target on the display (bottom-up search). In our previous work we showed that when using the spiking Search over Time and Space (sSoTS) model [2] with reduced saliency, we could simulate similar effect in the traditional bottom-up visual search experiment [3]. In our previous work we also predicted that similar affects can be observed in top-down visual search [3] and this outcome was confirmed in our behavioural studies.
To further investigate the relationship between target and distractor in the two groups and the differences amongst the groups we used traditional visual search experiment in both bottom-up and top-down search, where the target was bigger in size that the distractors. In the current work we present computational modelling studies where we investigate the underlying processes of the cross-cultural differences for bottom-up and top-down visual search using different size of target and distractors.
REFERENCES 1.Nisbett, R.E. and T. Masuda, *Culture and point of view.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**(19): p. 11163-11170.
2.Mavritsaki, E., et al., *Bridging the Gap Between Physiology and Behavior: Evidence From the sSoTS Model of Human Visual Attention.* Psychological Review, 2011. **118**(1): p. 3-41.
3.Mavritsaki, E. and P. Rentzelas, *Cross-cultural differences in visual attention: a computational modelling study.* BMC Neuroscience, 2015. **16**(1): p. 1-1.

Disclosures: E. Mavritsaki: None. **P. Rentzelas:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

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Program#/Poster#: 465.01/LLL65

Topic: H.02. Human Cognition and Behavior

Support: MOST 105-2420-H-002 -004 -MY2

Title: Emergence of distinct dynamical states in the brain network for self-other processing

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Abstract: Self-other processing, as a domain-specific function, has implications for understanding social cognition and some psychiatric conditions. Empirical research has confirmed the relevance of some anatomical structures, such as medial prefrontal cortex and temporo-parietal junction, in the processing of self-other-related information. Some recent studies pursued a finer-grained spatial demarcation of these structures into “self-specific” and “other-specific” sub-regions. This approach, however, overemphasizes the assumption of spatial localization and neglects the potential role of dynamical states as representational states. Other studies treated these structures as components of a dynamical system by analyzing their functional connectivity. Nevertheless, the seeds in these studies were usually chosen arbitrarily, leaving some room of ambiguity. Employing a data-driven approach, we registered the term-based meta-analytic results from the Neurosynth human fMRI database to the NTU-122 diffusion spectrum image template and applied end-to-end fiber tracking algorithm and hierarchical clustering to identify the vertices and edges, thus obtaining a network that maximally preserves the activation and connectivity pattern of self-other processing brain regions. We then implemented computational simulation to explore the synchronization spectrum and compared the results with those derived from an anatomically defined, arbitrary network. It revealed that the data-driven network has a higher probability of settling into some discrete states than does the arbitrary network. The co-existence of discrete limit sets within the same dynamical system suggests an alternative hypothesis of self-other processing to the conventional spatial localization argument.

Disclosures: **Y. Chen:** None. **T. Huang:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: Start-up funds provided by the University of Iowa to MD

Pilot scanning time provided by the University of Iowa Magnetic Resonance Research Facilities

Title: Brain networks associated with neuroticism, social networks, and loneliness in Traumatic Brain Injury

Authors: *J. N. BEADLE[1], A. RIGON[2], M. VOSS[3], M. DUFF[4];
[1]Gerontology, Univ. of Nebraska at Omaha, Omaha, NE; [2]Neurosci., [3]Psychological and Brain Sci., [4]Communication Sci. and Disorders, Univ. of Iowa, Iowa City, IA

Abstract: INTRODUCTION. Traumatic brain injury (TBI) can significantly impact cognition, emotion, and social behavior, as well as contribute to personality changes that may negatively affect social relationships. However, little is known about the impact of TBI on social network size and loneliness, and interactions with personality. Furthermore, by studying the brain networks supporting these processes, researchers can develop targeted interventions to improve social well-being in TBI. Thus, we investigated relationships between social network size, loneliness, and personality in TBI and examined brain networks supporting social relationships. METHODS. Participants included 22 individuals with chronic moderate-severe TBI and 19 demographically-matched healthy comparison participants (HC). Participants completed self-report questionnaires measuring perceived loneliness (UCLA loneliness scale), neuroticism (NEO-FFI), and social network size (NSHAP). Additionally, participants underwent resting state functional magnetic resonance imaging (rs-fMRI) to obtain a measure of functional connectivity (rs-FC). RESULTS. Individuals with TBI and HCs had comparable social network sizes ($p>.05$). Despite this fact, individuals with TBI had higher perceived loneliness ($p<.05$) and neuroticism ($p<.05$) than HCs. Within the TBI group, there was a strong, positive correlation between loneliness and neuroticism [$r(21)=.86$, $p<.001$], and significant, negative correlations between social network size and loneliness [$r(21)= -.53$, $p<.05$], and neuroticism [$r(21)= -.49$, $p<.05$]. A mediation analysis revealed that within the TBI group, loneliness had a significant indirect effect on social network size which was mediated by neuroticism [b=.-1.32, 95% BCa CI [-2.54, -.43]; large effect size, $k^2=.45$]. To examine the relationship between neuroticism and brain connectivity in TBI, we conducted an exploratory whole-brain analysis selecting the amygdala as a seed region. Individuals with TBI who reported higher neuroticism had less functional connectivity between amygdala and precuneus than HC. DISCUSSION. We found that despite having similar numbers of friends to healthy adults, patients with TBI felt lonelier, and high neuroticism mediated this relationship. Furthermore, in TBI patients, brain networks related to neuroticism involved regions important for social relationships (amygdala) and self-regulation (precuneus) in healthy adults. Taken together, the present study provides evidence that in TBI the brain networks supporting neuroticism may be an important target for rehabilitation efforts aimed at improving social well being.

Disclosures: J.N. Beadle: None. **A. Rigon:** None. **M. Voss:** None. **M. Duff:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: MIUR grant (PRIN 2010XPMFW4_008; I meccanismi neurocognitivi alla base delle interazioni sociali)

Title: Perceived social isolation is associated with altered functional connectivity in neural networks associated with tonic alertness and executive control

Authors: *E. LAYDEN[1], J. T. CACIOPPO[1], S. CACIOPPO[2], S. F. CAPPA[3], A. DODICH[4], A. FALINI[4], N. CANESSA[3];
[1]Psychology, [2]Psychiatry and Behavioral Neurosci., The Univ. of Chicago, Chicago, IL; [3]Inst. Universitario di Studi Superiori, Pavia, Italy; [4]San Raffaele Scientific Inst., Milan, Italy

Abstract: Perceived social isolation (PSI), colloquially known as loneliness, is associated with selectively altered attentional, cognitive, and affective processes in humans, but the neural mechanisms underlying these adjustments remain largely unexplored. Behavioral, eye tracking, and neuroimaging research has identified associations between PSI and implicit hypervigilance for social threats. Additionally, selective executive dysfunction has been evidenced by reduced prepotent response inhibition in social Stroop and dichotic listening tasks. Given that PSI is associated with pre-attentional processes, PSI may also be related to altered resting-state functional connectivity (FC) in the brain. Therefore, we conducted the first resting-state fMRI FC study of PSI. Five-minute resting-state scans were obtained from 55 participants (31 females). Analyses revealed robust associations between PSI and increased brain-wide FC in areas encompassing the right central operculum and right supramarginal gyrus, and these associations were not explained by depressive symptomatology, objective isolation, or demographics. Further analyses revealed that PSI was associated with increased FC between several nodes of the cingulo-opercular network, a network known to underlie the maintenance of tonic alertness. These regions encompassed the bilateral insula/frontoparietal opercula and ACC/pre-SMA. In contrast, FC between the cingulo-opercular network and right middle/superior frontal gyrus was reduced, a finding associated with diminished executive function in prior literature. We suggest that, in PSI, increased within-network cingulo-opercular FC may be associated with hypervigilance to social threat, whereas reduced right middle/superior frontal gyrus FC to the cingulo-opercular network may be associated with diminished impulse control.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 465.04/LLL68

Topic: H.02. Human Cognition and Behavior

Support: USC Zumberge Award

Title: All Together Now: The neural basis of social development associated with music training

Authors: ***M. SACHS**[1], B. ILARI[2], J. KAPLAN[1], H. DAMASIO[1], A. DAMASIO[1], A. HABIBI[1];
[1]Brain and Creativity Inst., Los Angeles, CA; [2]Thorton Sch. of Music, USC, Los Angeles, CA

Abstract: Playing music, particularly in an ensemble setting, requires a certain degree of social acuity in that the performer must learn to identify and match the emotional states of others. Despite this, while active musical engagement has been shown to be associated with enhanced cognitive capabilities that are rooted in structural and functional changes in the brain, the relationship between socio-emotional abilities, music education, and the brain remains largely unexplored. Using fMRI, and a novel, non-language based measure of emotional judgment, we investigated the behavioral and neural correlates of socio-emotional processing in young musicians who had received varying degrees of formal music training. Violinists between the ages of 8-12 were scanned while performing two tasks, one musical and one nonmusical, that involved inferring the emotional states of others. In the musical task, the participant either watched (video condition) or listened to (audio condition) a musical performance of a violinist and had to identify which of four emotions the performer was conveying. In the nonmusical task, the participant was presented with a face and had to identify the emotion that was being conveyed. Behavioral results showed that years of musical training was positively correlated with accuracy on identifying the emotion of the faces as well as identifying the emotion of the performer in the audio condition only. The fMRI results showed that the task conditions differentially activated regions known to be involved in distinguishing between emotions. Furthermore, in the audio only condition, children with more music training showed a greater difference in BOLD signal between positive and negative valence emotions in the parietal cortex (superior parietal lobule and post-central gyrus) and the prefrontal cortex (superior frontal gyrus and medial prefrontal gyrus). These regions belong to the action observation network, which underlies our ability to infer the intentions of other's actions. The results therefore suggest that in the absence of visual cues, children with more musical training are engaging part of this action observation network in order to distinguish between certain emotions. Such findings provide support for the notion that music education has social and emotional benefits and have

implications for our understanding of neurodevelopmental disorders in which emotional processing is attenuated.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: NHMRC APP1004740

Swinburne University Neuroimaging Grant

Title: Social Disorganisation links autism and schizophrenia spectrum disorders through excitation-inhibition neurotransmitter imbalance.

Authors: ***T. C. FORD**[1], R. NIBBS[2], D. P. CREWTHER[1];
[1]Ctr. for Human Psychopharmacology, [2]Brain and Psychological Sci. Res. Ctr., Swinburne Univ. of Technol., Melbourne, Australia

Abstract: The phenotypes of autism and schizophrenia spectrum disorders show an overlap both in the clinical and non-clinical population, which has been conceptualized as Social Disorganization (SD). In this study, the glutamate/gamma-aminobutyric acid (GABA) concentration ratio between high and low scorers on SD was investigated. A low (female: n=10, age=22.90(4.15); male: n=8, age=25.75(7.13)) and high (female: n=20, age=23.50(6.65); male: n=10, age=22.20(4.61)) SD scoring group aged 18 to 40 years underwent proton magnetic resonance spectroscopy (^{1}H-MRS) for glutamate and GABA concentrations in two superior temporal voxels of right and left hemisphere. Glutamate spectra were isolated from glutamine and GABA using a PRESS sequence with an echo time of 80ms, while MEGA-PRESS was used to isolate the GABA spectra. Separate 2 (hemisphere) by 2 (group) mixed ANOVAs were conducted for glutamate level, GABA level and glutamate/GABA ratio followed by *post hoc* analyses. Reduced GABA level ($t(20.70) = -2.93$, $p = 0.008$, Hedge's $g = 1.05$) and increased glutamate/GABA ratio ($t(29.21) = 3.88$, $p < 0.001$, Hedge's $g = 1.35$) was found in the right temporal voxel for the high SD group. In the left temporal voxel, GABA was significantly higher for the high SD group ($t(32.59) = 2.26$, $p = 0.031$, Hedge's $g = 0.73$). Results point toward an excess excitation to inhibition imbalance in the right superior temporal region as a function of

reduced inhibition in this region for those with high scores on the shared autism and schizophrenia spectrum phenotype SD.

Disclosures: **T.C. Ford:** None. **R. Nibbs:** None. **D.P. Crewther:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

Location: Halls B-H

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Program#/Poster#: 465.06/LLL70

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 330989; CVU 619655

Title: Psychotherapists show significant differences in perspective taking, emotional regulation and brain network connectivity

Authors: *V. E. OLALDE[1], S. ALCAUTER, 76230[2], F. BARRIOS, 76230[2], R. MERCADILLO[3], S. FEDERICA[2], E. PASAYE[2];
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Abstract: There is recent evidence of the cognitive modulation of empathy, which is of great interest to delineate strategies to improve social interactions and reduce conflicting behaviors, such as bullying. However, little is known about the neural substrate of the components of the empathic response and how they can be modulated with training. To get more understanding of these interactions we characterized a group of alliance-therapists which long-term training is based in the frequent modulation of such empathic components, including perspective taking and emotional regulation. We contrasted behavioral scores and resting state functional connectivity (FC in a group of therapists and non-therapists. The behavioral scores of the perspective taking and the emotional regulation components were measured by the interpersonal reactivity index and the emotional regulation questionnaire, in a sample of 52 therapists (mean age 49.3y, 22 males) and 62 controls (mean age 51.7y, 36 males). For the resting state fMRI study two groups of 10 therapists (mean age 55.8y, 3males) and 10 controls (mean age 55.7y, 3 males) were included, they were paired by economic status, years of formal studies, age and sex. Both groups had more than 13y of professional experience
The resting state datasets were acquired with a 3T scanner, while subjects were awake with eyes closed. After standard preprocessing (no global signal regression), five noise based time series (CompCor) and movement affected volumes were regressed out. Spherical seeds located in the right Temporal Parietal Junction (TPJ), right middle TPJ and Anterior Insula were used to

generate corresponding connectivity maps. After Fisher Z transformations, two group t-tests were applied to identify differences between groups.
The therapists showed significantly higher scores in perspective taking, and significantly lower scores in emotional suppression. In addition, therapists showed less FC between posterior areas of the brain, involved with inference process and autobiographical retrieving. They also showed higher FC with ventral areas involved in language processing and emotion regulation. In sum, these results suggest that the therapists use different strategies to infer the mental state of others, and that these strategies can be represented in the FC between different networks. Although, we don't know if such differences were present before training and predispose subjects for such profession, these results warrant further research on the effects of shorter training strategies.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Program#/Poster#: 465.07/MMM1

Topic: H.02. Human Cognition and Behavior

Support: Stiftelsen Söderström-Königska sjukhemmet

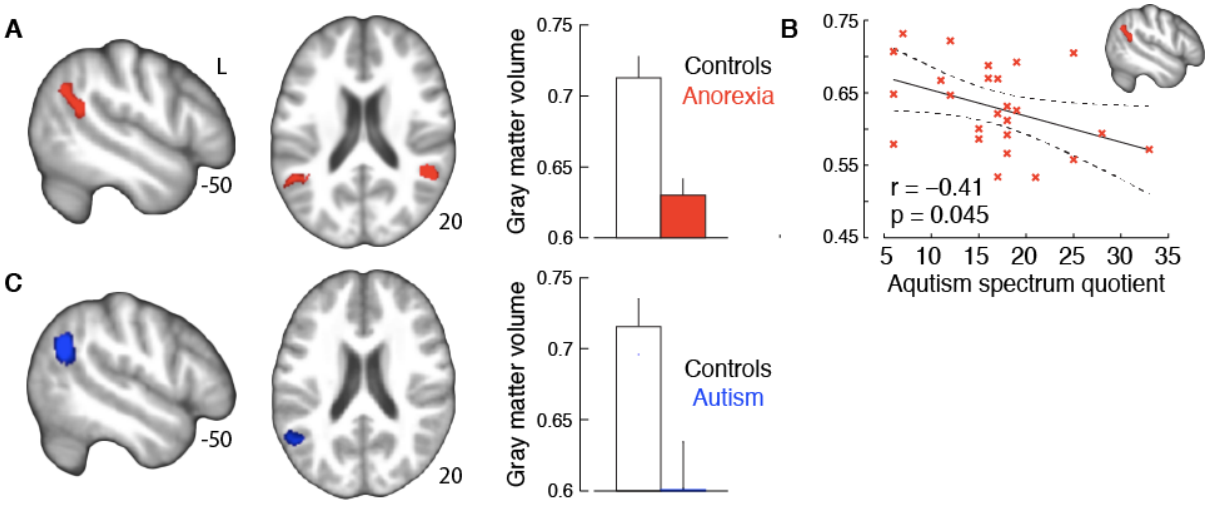
ALF-Västra Götaland

Title: Shared cortical alterations in anorexia nervosa and autism spectrum disorder

Authors: *M. BJORNSDOTTER[1,3], M. DAVIDOVIC[2], L. KARJALAINEN[2,4], G. STARCK[2], H. OLAUSSON[3], E. WENTZ[2];
[1]Ctr. for ethics, law and mental health, Univ. of Gothenburg, Goeteborg, Sweden; [2]Univ. of Gothenburg, Gothenburg, Sweden; [3]Ctr. for Social and Affective Neurosci., Linköping Univ., Linköping, Sweden; [4]Anorexia-Bulimia Unit, Queen Silvia Children's Hosp., Sahlgrenska Univ. Hosp., Gothenburg, Sweden

Abstract: Converging findings point to a considerable overlap in cognitive and behavioral traits between anorexia nervosa (AN) and autism spectrum disorder (ASD): women with AN exhibit perfectionism, particularly regarding symmetry and exactness (Srinivasagam et al. Am J Psychiatry, 1995:152) and impaired social cognition (Cassin et al. Clinical Psychology Review, 2005:25; Zucker et al. Psychol Bull, 2007:133). Here, we asked whether the two conditions share cortical alterations. We acquired structural magnetic resonance imaging (MRI) scans in women

diagnosed with AN (n = 25) and matched control women (n = 25), as well as in all age and gender-matched participants with an ASD diagnosis (n = 14) and control participants (n = 22) of the Autism Brain Imaging Data Exchange. Regional gray matter volume alterations were assessed using voxel-based morphometry (VBM). Women with AN scored higher on the Autism spectrum Quotient (AQ) than the control group (p = 0.010). AN patients exhibited significantly reduced gray matter volume of bilateral superior temporal sulcus (STS), extending into the temporoparietal junction (TPJ), relative to control participants (p<0.005, k≥247)(Figure A). AQ correlated significantly with average gray matter volume of the left, but not right, STS region in women with AN (r = -0.41, *p* = 0.045)(Figure B). Women with ASD exhibited significantly reduced gray matter of the left STS region compared to control participants (p<0.005, k≥256)(Figure C). The same left STS region was affected in both AN and ASD: women with AN exhibited reduced average gray matter of the left STS region identified in the ASD group comparison (p=0.002), and women with ASD showed significantly reduced volume of the left (p=0.027) region identified in the AN group comparison. Our results show that women with AN and ASD share cortical alterations in the form of reduced gray matter of a key brain structure involved in social cognition, supporting the previously suggested link between the two conditions (Gillberg, 1983:142).



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Poster

465. Neural Processes and Disorders of Social Cognition

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NHMRC Senior Research Fellowship APP1103258

Title: Divergent facial scanning patterns in behavioural-variant frontotemporal dementia and semantic dementia

Authors: *R. HUTCHINGS[1,2,3], R. PALERMO[4,3], J. BRUGGEMANN[2,1], J. R. HODGES[1,2,3], O. PIGUET[1,2,3], F. KUMFOR[1,2,3];
[1]Neurosci. Res. Australia, Randwick, Australia; [2]Univ. of New South Wales, Sydney, Australia; [3]ARC Ctr. of Excellence in Cognition and its Disorders, Sydney, Australia; [4]Univ. of Western Australia, Crawley, Australia

Abstract: Faces offer an incredible wealth of information for social interactions. Emerging evidence suggests that some clinical groups (e.g., autism) with impaired emotion recognition do not appropriately attend to parts of the face that display emotion (e.g., eyes). Two variants of frontotemporal dementia (FTD): behavioural-variant frontotemporal dementia (bvFTD) and semantic dementia (SD), have frontal and/or temporal atrophy, which overlaps with brain networks involved in face and emotion processing. Existing evidence has shown that both bvFTD and SD patients have behavioural deficits in labelling facial expressions. Whether this impairment is due to inappropriate facial scanning, however, is unknown. This study investigated facial scanning in 20 bvFTD, 12 SD and 21 control participants. Eye tracking was recorded while participants passively viewed faces across 72 trials (3 blocks of 8 fearful, 8 happy, 8 neutral faces). Results revealed a significant group difference in the number of fixations to the eyes during the first block ($p = 0.044$). Specifically, bvFTD participants showed more fixations to the eyes than controls in both the happy ($p = 0.046$) and fearful ($p = 0.023$) condition. Furthermore, bvFTD patients showed more fixations to the eyes in the fearful condition than the SD group ($p = 0.048$). This difference diminished over repeated exposure to stimuli (i.e., across blocks), with all groups looking less at the eyes with repeated viewings of the face ($p < 0.005$). These results indicate that despite evidence of emotion labelling deficits, both bvFTD and SD are looking at emotionally relevant face regions. In particular, bvFTD participants look more at they eyes than healthy controls when viewing emotional faces, which may be suggestive of an attempt to compensate for deficits. Differences in facial scanning between these subtypes of FTD suggest distinct underlying processes influencing facial emotion decoding. Future analyses will identify the neural correlates of facial scanning patterns in these dementia syndromes. Overall, our results

provide impetus for further investigation into the mechanisms of face and emotion processing in these FTD syndromes, which will, in turn, inform our knowledge of the face-processing network.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Wellcome Trust Investigator Award 098362/Z/12/Z

Title: Neural and computational processes underlying dynamic changes in self-esteem

Authors: *G.-J. WILL[1], R. B. RUTLEDGE[1,2], M. MOUTOUSSIS[1], R. J. DOLAN[1,2]; [1]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom; [2]Max Planck Univ. Col. London Ctr. for Computat. Psychiatry and Ageing Res., London, United Kingdom

Abstract: Onset of psychiatric disorders is often preceded by a decline in self-esteem, and this is thought to be related to a heightened sensitivity to negative evaluation from peers. The mechanisms through which repeated negative evaluation leads to declines in self-esteem are largely unknown. To characterize the neural and computational processes underlying changes in self-esteem, we asked healthy young adults to perform a task in which they received acceptance and rejection feedback from peers who evaluated them based on an online profile. Participant expectations were manipulated by sorting peers into four groups based on the overall likelihood of giving positive social feedback to other participants. On each trial, participants were provided with a visual cue that indicated which group a peer belonged to and predicted whether the peer would like or dislike them before receiving acceptance or rejection feedback. After every 2-3 trials, participants reported their current level of self-esteem. Using computational modeling, we found that moment-to-moment fluctuations in self-esteem were best explained by the combined influence of: 1) recent expectations about social feedback and 2) social prediction errors arising from those expectations upon receipt of feedback (i.e. the difference between actual and expected feedback). Using functional magnetic resonance imaging, we show that social

prediction errors correlate with activity in the ventral striatum and momentary changes in self-esteem co-vary with activity in ventromedial prefrontal cortex. These findings provide insight into the computations performed in the brain during social feedback processing and the likely mechanisms through which social feedback cumulatively impacts on self-esteem. These results are relevant to understanding why volatility in self-esteem is a vulnerability factor for mental health problems.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER DRL-0746970

NSF Spatial Intelligence and Learning Center

TFK Foundation

NCER R305C050076

Title: A neural correlate of math anxiety in the ventromedial prefrontal cortex

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Abstract: Many individuals report strong feelings of tension, apprehension, and fear of math (Richardson and Suinn, 1972) as exemplified by the fact that 30% of students feel helplessness when solving math problems (OECD, 2013). This emotional reaction, termed Math Anxiety (MA), has long been associated with avoidance of math and poor math performance (Hembree, 1990), which may be depriving societies of individuals with the mathematical expertise needed for economic and technological development (National Mathematics Advisory Panel, 2008). The underlying neurocognitive mechanisms of math anxiety remain unclear (Artemenko et al., 2015), hindering the development of effective preventions and interventions. Using functional magnetic resonance imaging (fMRI), we investigated how MA modulates brain activity while nineteen participants were solving easy and hard math problems. MA is more pronounced when solving hard math problems (Ashcraft and Kirk, 2001; Lyons and Beilock, 2012), therefore we focused

on the difficulty-related activation by contrasting easy and hard trials. SPM12 was used to preprocess and analyze fMRI data, including motion correction, coregistration, spatial normalization into the MNI space (voxels resampled to 2 x 2 x 2 mm), spatial smoothing (6 mm FWHM Gaussian kernel), and statistical modeling of each participant. Difficulty-related activation was related to MA using a group-level multiple regression model, in which the participants' MA scores, age, and gender were entered as covariates. To identify significant voxel clusters, a non-parametric permutation test was performed using SnPM13 (Nichols and Holmes, 2001) with a cluster-forming threshold equivalent to $p < 0.001$ at the voxel level. The analysis revealed only one cluster (x, y, z = 0, 42, -14; $k = 169$; $T(15) = 7.16$; P [FWE] = 0.019) in the ventromedial prefrontal cortex (vmPFC). Consistent with previous research (Young et al., 2012), the vmPFC deactivated in hard relative to easy math trials, and MA was positively associated with the level of vmPFC deactivation. Interestingly, this part of vmPFC has been shown to deactivate when people judged trait adjectives to be less self-descriptive or less important to oneself (D'Argembeau et al., 2012), indicating a plausible link between MA and distancing one's self-representation from math (Necka et al., 2015). When faced with difficult math tasks, the higher one's MA, the more they may distance their self-concept from math.

Disclosures: K. Choe: None. **A. Mattarella-Micke:** None. **M.G. Berman:** None. **S.L. Beilock:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: Singapore Ministry of Education Academic Research Fund (Tier 2: MOE2012-T2-1-051)

Title: When cultures mix: neurobehavioral responses to the visual mixing of cultural symbols

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Abstract: Recent work in cultural psychology suggests that humans represent the intrusion of foreign cultures using mechanisms similar to contamination detection and rejection. Similarly, social neuroscience studies have identified brain areas that signal violations of social norms or

order. Here, we examine how the human brain represents the visual depiction of cultural mixing. We developed images where cultural symbols from the home culture were mixed with symbols from a foreign culture. These images were employed in an N-back task, without any further instructions. Neuroimaging results suggest the involvement of amygdala in the response to mixing with foreign cultural symbols (but not when mixing with same-culture symbols). Specifically, bilateral amygdala responses (paired ttest p(fwe) = .014; region of interest analysis using bilateral amygdala) were higher when the local cultural symbol was mixed with the foreign cultural symbol, as compared when it was mixed with another local symbol. These preliminary results suggest that the human brain is sensitive to cultural symbols, especially when they are mixed with symbols representing foreign cultures. These results could help us understand how humans respond to an increasingly multicultural world.

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Poster

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Title: Excessive association between negative intentionality and immorality is diminished in autism spectrum disorder

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Abstract: Moral judgment is a highly complex mental process, and the features that drive it have been the center of research in psychology, neuroscience, and philosophy. Classical models assumed that intentionality of an action guides moral judgments on that action. However, recent findings suggest that moral judgment could inversely affect judgment on intentionality. Specifically, most people attribute intentionality to morally bad side-effects, but not to morally good side-effects. In the present study, we focused on this phenomenon, known as 'the Knobe effect', and examined the psychological and neural mechanisms underlying this effect using functional magnetic resonance imaging (fMRI) by comparing adults with autism spectrum disorder (ASD) and neurotypical adults (NT). We prepared various types of scenarios, including not only negative but also positive scenarios, such as intended, attempted, and accidental harms/helps. Furthermore, we prepared two distinct sets of 20 negative and 20 positive side-effects in contrast that previous studies examined the Knobe effect with only few typical scenarios, so that we could quantify the effect and eliminate scenario-specific factors. Thirteen ASD and 11 NT judged the degree of morality and intentionality of the protagonist's action for each scenario in the scanner. We found that both ASD ($P = 0.02$) and NT ($P < 0.001$) showed significant Knobe effects. Interestingly, attribution of intentionality in negative side-effects was significantly smaller in ASD than NT ($P = 0.03$), with no significant difference in positive side-effects, indicating that the Knobe effect is attenuated in ASD. In addition, moral judgments for attempted harms were less severe in ASD than NT ($P < 0.05$). We hypothesized that this decreased dependence of moral judgment on intentionality is related to the diminished Knobe effect in ASD. We quantified how much moral judgment of each individual depends on intentionality (the degree of intentionalism: DOI) by comparing moral judgments for attempted helps to attempted harms. We could derive DOI from these scenarios, since protagonists brought the same morally neutral outcomes with either good or bad intentions there. Interestingly, as with the Knobe effect, DOI was significantly lower in ASD than NT ($P < 0.05$). Moreover, DOI showed a significant positive correlation with the strength of the Knobe effect ($P < 0.001$), that is, those who depends more on intentionality in moral judgment showed stronger Knobe effects. These results suggest that the Knobe effect is subserved by a bidirectional interaction between intentionality and morality, and help to understand the social difficulties in people with autism.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Program#/Poster#: 465.13/MMM7

Topic: H.02. Human Cognition and Behavior

Title: Neural responses to moral violations do not support a division between individualizing and binding categories

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Abstract: Moral Foundations Theory (MFT) is the predominant social psychological explanatory framework for the recurrence of moral themes across cultures, decomposing morality into the canonical foundations of authority, loyalty, purity, fairness, and harm. Recent additions to this framework have proposed a liberty foundation. These themes have been theoretically united under superordinate headings of "individualizing" foundations (harm and fairness), which center on wrongs perpetrated against an individual; and "binding" foundations (authority, loyalty, and purity), which center on wrongs perpetrated against the community. To date, no neuroimaging study of morality has examined the entire set of moral violations proposed by the MFT. The present study used functional magnetic resonance imaging (fMRI) to investigate the neural correlates underlying each of the proposed moral foundations, and to probe whether the superordinate categories of individualizing and binding are appropriate ways to dissociate morality at the neural level. While in the scanner, participants provided judgments of the moral wrongness of actions which violated the canonical foundations (including liberty) as well as violations of nonmoral social norms. Analysis of the imaging data revealed that almost all violations deemed morally wrong elicited activity from a set of regions comprising the default mode network. Regions included the dorsomedial prefrontal cortex, and medial posterior cortical regions, extending across retrosplenial and posterior cingulate cortex as well as precuneus. Exceptions to this activation pattern were found in violations of the authority foundation and violations of the harm foundation when the harm was physical as opposed to emotional. Additionally, violations of fairness and loyalty both elicited bilateral activity in ventrolateral parietal cortical areas and medial prefrontal cortices, potentially reflecting foundation-level differences in attentional allocation and/or mentalizing respectively. Collectively, these findings support the idea that it may not be appropriate to unite foundations under the headings of individualizing and binding when discussing the neural substrates of morality.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Singapore Ministry of Education Academic Research Fund (Tier 2: MOE2012-T2-1-051)

Title: A neuroimaging account of accountability in allocentric decision making

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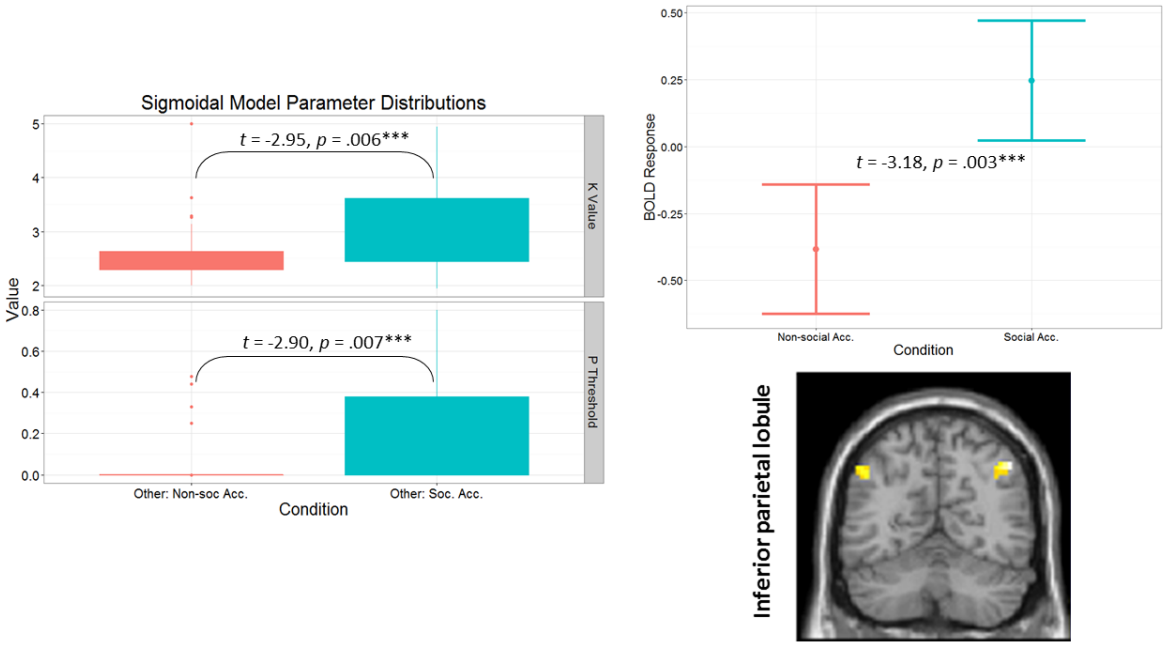
Abstract: Neural mechanisms of subjective reward valuation are well established in the literature; however little research has explored neurological mechanisms of vicarious reward valuations. This occurs when decision makers choose outcomes for recipients other than themselves and have no vested interest in the outcomes. We refer to these scenarios as allocentric decisions. One important control mechanism on allocentric decision making is accountability from external regulators. Here, we examine the role of accountability as participants made choices for another about a typing task with decision trade-offs between a task's effort and monetary reward (Masser et al. 2015). Using fMRI, we examined the neural processes of participants making both socially accountable and private decisions within-subjects. Behavioral analyses show socially accountable decisions discounted effort more than non-social accountability choices and resulted in more low-effort low-reward outcomes. Fitting the behavioral data with a sigmoidal model of effort discounting shows an increase in both the discounting rate (t_k = -2.95, p_k = 0.006) and the threshold level at which discounting begins (t_p = -2.90, p_p = 0.007) under social accountability. Preliminary neuroimaging analysis identified bilateral activation of inferior parietal lobule (IPL) when contrasting decision making under social accountability compared with non-social accountability (48, -61, 47; $p_{(unc)}$ = .002; -45, -58, 38; $p_{(unc)}$ = .007). This brain area has been repeatedly associated with self-other discrimination and social cognition more generally (Uddin et al. 2006). These results could further

understanding of how social controls - intrinsic to organizational environments - could affect decision mechanisms.



Disclosures: **S. Fitzgerald:** None. **G. Christopoulos:** None.

Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: KAKENHI 15H05875

KAKENHI 15K12775

KAKENHI 15H01846

Title: Over-entrainment to the partner’s eye-blinks during eye contact in autistic spectrum disorders

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Abstract: It has been proposed that the difficulty in shifting mental set, which is critical in social interaction, may be the root of cognitive impairment of persons with autism spectrum disorders (ASD). ASD have difficulty in joint attention: sharing attention with social partners towards third object. As an initial eye contact prior to looking at an object is a critical cue for establishing joint attention, eye contact and joint attention are tightly coupled. We hypothesized that the impairment in shifting mental set is reflected in the train of eye contact alternated with joint attention task. Because joint attention task forces to synchronize the temporal attentional window demarked by eye-blink (Koike et al. 2016), the mental set is to be casted to the task. As interleaved eye contact does not require specific task, mental set should be shifted.
To test the hypothesis, we analyzed the video-recorded eye-movement including blink previously obtained during block-design hyperscanning fMRI with joint attention task by 20 pairs, i.e. ASD paired with typically-developed (TD) subjects, and 26 TD-TD pairs (Tanabe et al. 2012). In joint attention blocks (JA), one subject shifts own gaze toward the object on the screen. At the same time, other subject follow partner' gaze shift to share their attention on the same object. In eye contact blocks (EC) both subjects keep eye contact. The noise contribution ratio (NCR), the measure of influence, was evaluated with a multivariate autoregressive model. As a baseline of NCR, we calculated the non-pair-NCR across all possible combination of subjects and blocks. The ΔNCR, enhancement of NCR from the baseline, was used as a degree of influence of eye blink from the partner.
During EC, ΔNCR was negatively correlated with verbal IQ (VIQ) both in ASD and TD. After modeling out the variance due to the VIQ, two-way ANOVA of condition [EC, JA] × group [ASD, TD] showed the significant main effect of condition ($F(1,25)=10.03$, $p=0.004$) and interaction ($F(1,38) = 4.929$, $p=0.036$). The interaction was not significant in TD-TD group. That is, partner's eye blink influenced own eye blink during EC, more prominent in ASD than TD. As JA tasks were strictly identical across the block and subjects, partner-specific influence was minimized. During EC, no specific task was requested, thus partner's influence became evident which may represent the carry-over of the JA task-specific influence from the partner. Furthermore, the degree of influence was negatively correlated to the VIQ reflecting executive function. Thus the over-entrainment of the ASD conceivably reflects their impaired executive function of shifting mental sets.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Topic: H.02. Human Cognition and Behavior

Support: National Institute on Drug Abuse (R01 DA036028, R21 DA024419)

Annenberg Public Policy Center of the University of Pennsylvania

Title: Connectivity between auditory and visual cortices mediates the impact of argument strength on the efficacy of anti-smoking videos among low-sensation-seeking smokers

Authors: *Z. SHI[1], V. FAIRCHILD[1], A.-L. WANG[1], C. ARONOWITZ[2], J. N. CAPPELLA[3], D. ROMER[2], D. D. LANGLEBEN[1];
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Abstract: Smoking-cessation videos with high argument strength (AS) are more effective at reducing smoking behavior than low-AS ones. Prior research suggested that the interaction between AS and sensory aspects of the video messages are associated with their overall effectiveness. In addition, studies agree on the importance of the sensation-seeking trait in smokers' sensitivity to smoking-cessation interventions. We hypothesized that integration of visual and auditory (i.e. multisensory) processing would mediate the effectiveness of high-AS smoking-cessation videos on subsequent smoking behavior, and that such mediation would be moderated by smokers' sensation seeking. Using functional magnetic resonance imaging (fMRI), we recorded the brain response of 66 smokers (32 females) randomly assigned to view either 16 high-AS or 16 low-AS smoking-cessation videos. A validated I² content analytic measure (Lang et al. 2006) quantified the amount of visual and auditory information in each video, which was matched between high-AS and low-AS videos. Multisensory processing of the videos was indexed by the functional connectivity between sensory cortices. Specifically, such connectivity was assessed by identifying cortical regions whose activity was parametrically modulated by the visual and auditory I² scores, and computing the correlation between fMRI signals extracted from these regions. Sensation seeking was measured using the Brief Sensation Seeking Scale (Hoyle et al. 2002). Smoking behavior was assessed with urine levels of nicotine metabolite cotinine immediately before (baseline) and approximately 30 days after (follow-up) the fMRI session. We tested a moderated mediation model using AS as the input variable, follow-up cotinine level (adjusted for baseline) as the outcome variable, multisensory neural connectivity as the mediator, and sensation seeking as the moderator. We found a significant (p=.01) moderated mediation effect: the high-AS videos produced greater connectivity, which in turn negatively predicted follow-up cotinine levels among the low-sensation-seeking smokers. This

effect was absent in the high-sensation-seeking group. Our results suggest that 1) audiovisual neural integration underlies the greater efficacy of high-AS smoking-cessation videos, and that 2) high sensation seeking confers resistance to anti-smoking arguments. These findings highlight the importance of accounting for brain connectivity and individual differences in the evaluation of anti-smoking messaging in particular and public health communication in general.

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Poster

465. Neural Processes and Disorders of Social Cognition

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Hope for Depression Research Foundation

NIMH (5R01MH073719-06)

NIDCD (5R01 DC004290-14)

NINDS (1R01NS088748-01)

Title: Affective bias is sensitive to acute electrical stimulation of two limbic regions: subcallosal cingulate white matter and amygdala

Authors: *K. C. ROWE[1], H. S. MAYBERG[2], C. K. KOVACH[5], C. S. INMAN[3], A. L. CROWELL[2], R. E. GROSS[3], D. L. DRANE[4], J. T. WILLIE[3];
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Abstract: Background: Electrical brain stimulation is a currently being evaluated as an experimental therapy for depression, as well as being investigated as a method for mapping emotional brain functions. As deep brain stimulation (DBS) expands in the realm of psychiatric disorders, sensitive measures are needed to quantify effects of stimulation on emotional processing. We examined the effects of acute stimulation to two limbic regions, the subcallosal cingulate (SCC) and the amygdala, on affective bias in the perception and evaluation of

emotional facial expressions. We hypothesized that transient electrical stimulation to the limbic system would produce acute reductions in negative bias. **Methods:** A novel affective bias task was developed to quickly and implicitly measure emotional state. Over 4-6 minutes, patients rate the intensity and valence of images of emotional facial expressions. We examined stimulation effects in two groups: patients with treatment-refractory depression undergoing SCC DBS therapy, and epilepsy patients undergoing amygdala stimulation via stereo-EEG electrodes during inpatient intracranial monitoring. Patients completed the task during high-frequency stimulation of the SCC or amygdala, and during sham stimulation conditions. **Results:** Three SCC DBS patients showed significant positive shifts in affective bias with chronic DBS therapy. Furthermore, two DBS patients showed a rapid negative shift in bias following acute (18 minutes) discontinuation of chronic stimulation. Likewise, seven epilepsy patients show significantly positive shifts in affective bias with acute amygdala stimulation. **Discussion:** Affective bias shows rapid, significant changes with stimulation at one white matter limbic target and one gray matter limbic target, suggesting utility as an emotional outcome measure in brain stimulation studies. This task may facilitate tracking and predicting treatment response in DBS therapy. Future studies will determine whether affective bias can predict possible neuropsychiatric complications in patients undergoing mapping of brain circuitry ahead of resection surgery for epilepsy.

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Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Topic: H.03. Schizophrenia

Support: MH-077851

MH-078113

MH-077945

MH-077852

MH-077862

Title: Neural complexity as a potential translational biomarker for psychosis

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Abstract: Background: The adaptability of the human brain to the constantly changing environment is often reduced in patients with psychotic disorders, leading to impaired cognitive functions. Brain signal complexity, which may reflect adaptability, can be readily quantified via resting-state functional magnetic resonance imaging (fMRI) signals. We hypothesized that resting-state brain signal complexity is altered in psychotic disorders, and is correlated with cognitive impairment.
Methods: We assessed 156 healthy controls (HC) and 330 probands, including 125 patients with psychotic bipolar disorder (BP), 107 patients with schizophrenia (SZ), 98 patients with schizoaffective disorder (SAD) and 230 of their unaffected first-degree relatives (76 BPR, 79 SADR, and 75 SZR) from four sites of the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP) consortium. Using multi-scale entropy analysis, we determined whether patients and/or relatives had pathologic differences in complexity of resting-state fMRI signals toward regularity (reduced entropy in all time scales), or toward uncorrelated randomness (increased entropy in fine time scales that decays as the time scale increases) and how these complexity differences might be associated with cognitive impairment.
Results: Compared to HC subjects, all proband groups showed significantly increased brain signal randomness in dorsal and ventral prefrontal cortex (PFC), and unaffected relatives showed no complexity differences in PFC regions. SZ had the largest area of involvement in both dorsal and ventral PFC. BP and SAD probands shared increased brain signal randomness in ventral medial PFC, BP and SZ probands shared increased brain signal randomness in ventral lateral PFC, whereas SAD and SZ probands shared increased brain signal randomness in dorsal medial PFC. Only SZ showed increased brain signal randomness in dorsal lateral PFC. The increased brain signal randomness in dorsal or ventral PFC was weakly associated with reduced cognitive performance in psychotic probands.
Conclusion: These observations support the loss of brain complexity hypothesis in psychotic probands. Furthermore, we found an overlap of pathologic brain signal complexity between psychotic probands by DSM diagnoses, thus providing a biological basis for categorizing psychosis based on functional neuroimaging data.

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Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Topic: H.03. Schizophrenia

Support: XX00000

Title: Perineuronal net-associated extracellular matrix clusters in the normal human amygdala: relevance to SZ

Authors: *K. T. PILOBELLO[1], H. PANTAZOPOULOS[2], S. BERRETTA[2], R. MCCULLUMSMITH[3], S. ODONOVAN[3], R. KOENE[3], P. W. TILLBERG[4], E. S. BOYDEN[4]; [2]Mailman Res. Ctr., [1]McLean Hosp., Belmont, MA; [3]Col. of Med., Univ. of Cincinnati, Cincinnatti, OH; [4]Media Lab., MIT, Cambridge, MA

Abstract: Recent findings from our group show marked abnormalities affecting distinct components of the extracellular matrix in subjects with schizophrenia (SZ) and bipolar disorder (BD). In particular, perineuronal nets (PNNs) and glial cells labeled with markers for chondroitin sulfate proteoglycans (CSPGs) were altered in several brain regions in these disorders. The interactions between perineuronal nets and glia are multifold, as glial cells significantly contribute to making perineuronal nets and intimately regulate synaptic functions and plasticity. In the human amygdala, CSPGs bearing the CS-6 sulfation pattern form larger CSPG aggregates, named here 'CS-6 clusters', encompass several glial cells and are in close spatial relationship with PNNs labeled with the same markers. Subjects with SZ and BD showed profound decreases of both CS-6 clusters and CS-6 perineuronal nets. In the context of their relevance to these disorders, we tested the hypothesis that CS-6 clusters may represent conglomerates of perisynaptic CSPG aggregates within specialized multicellular domains.
In healthy human amygdalas (n= 3), CS-6 clusters were laser dissected and co-immunoprecipitated with CS-6 antibody, followed by mass spectrometry (MS) to identify proteins specific to CS-6 clusters. Confocal microscopy using CS-6 antibodies in combination with glial markers (glial fibrillary acidic protein (GFAP)) and a combination of expansion microscopy (ExM) and confocal microscopy were used to examine the relationship between CS-6 clusters, glial cells, PNNs and synapses.
The MS results show the CSPG versican as a major component of CS-6 clusters. Versican, involved in PNN formation, has splice-forms with developmentally regulated subunits of different CS-6 levels. Confocal microscopy shows that CS-6 clusters range in size (60-100 microns) and present with a pattern punctate labeling reminiscent of synaptic cartridges, radiating from their center. They are surrounded by glial fibrillary acidic protein (GFAP)-positive glia, while these cells are more sparsely distributed inside the clusters. CS-6-labeled

PNNs directly connect with the clusters. Images of CS-6 clusters using ExM, confirm this finding and are currently being used to test spatial relationship between punctate CS-6 condensations with the cluster and synapses.
Our results are consistent with the hypothesis that CS-6 clusters represent functional structures related to synaptic function and interact with PNNs, as well as subpopulations of glial cells. In SZ and BD, reductions of CS-6 clusters may represent specialized forms of extracellular matrix contributing to the synaptopathology of these disorders.

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Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Topic: H.03. Schizophrenia

Support: Canadian Institutes of Health Research

Title: Proteomic signatures associated with cognitive deficits in schizophrenia

Authors: *J. LAVOIE, C.-H. NA, L. SHAFFER, R. WARD, D. SCHRETLEN, K. ISHIZUKA, A. PANDEY, A. SAWA;
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Abstract: The discovery of novel molecular signatures that may represent biomarker candidates is essential to understand the pathophysiology of schizophrenia (SZ). Cognitive deficit is a key feature of SZ and a predictor of functional outcome in patients. However, the molecular signatures associated with cognitive impairment are not yet well understood. Recent major advances in human cell technologies have provided us opportunities to use neuronal cells from patients, such as olfactory epithelium (OE)-derived neuronal cells, that show immature neuronal traits and have been enriched to near homogeneity from biopsied OE. In the present investigation, we aimed to describe proteomic signatures in OE-derived neuronal cells from SZ patients and study their association with cognitive deficits. To do so, we have performed a pilot study with mass spectrometry using TMT labeling with OE-derived neuronal cells taken from age-, gender-, race- and smoking status-matched 10 controls and 10 SZ patients. Neuropsychological assessments were conducted for all subjects. The investigated domains include: full scale IQ, processing speed, verbal memory, visual memory, ideational fluency and

executive functioning. Among 7,179 quantified proteins, we reported 71 proteins differentially expressed between SZ patients and controls (q-value<0.05). Several KEGG pathways were found to be enriched, including the DNA replication pathway. Additionally, we found significant ($p<0.05$) differences in performance across neurocognitive tests with patients' performance being worse than controls for processing speed and ideational fluency, as well as trends ($p<0.1$) for lower IQ and verbal memory in SZ patients. The composite score was also significantly lower in SZ patients ($p=0.019$). The association between the differentially expressed proteins and the cognitive deficits in SZ was further investigated and several positive correlations were reported. These observations suggest that some proteomic signatures measured in OE-derived neuronal cells are associated with cognitive deficits in SZ. We believe that our unique approach of combining two observations from the same subject - protein expression and neuropsychological assessment - will help identifying new drug targets by connecting molecular phenotypes and clinical outcomes.

Disclosures: J. Lavoie: None. **C. Na:** None. **L. Shaffer:** None. **R. Ward:** None. **D. Schretlen:** None. **K. Ishizuka:** None. **A. Pandey:** None. **A. Sawa:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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NIH Grant AG027342

Investigator Initiated Grant from Janssen Scientific Affairs, LLC

Title: Abnormal trajectory of intracortical myelination in schizophrenia implicates white matter in disease pathophysiology and the therapeutic mechanism of action of antipsychotics.

Authors: *T. A. TISHLER[1], G. BARTZOKIS[1], P. H. LU[1], E. P. RAVEN[2], M. KHANOYAN[1], C. KIRKPATRICK[1], M. PYLE[1], P. VILLABLANCA[1], L. L. ALTSHULER[1], J. MINTZ[3], J. VENTURA[1], L. CASAUS[1], K. L. SUBOTNIK[1], K. H. NUECHTERLEIN[1], B. M. ELLINGSON[1];
[1]UCLA, Los Angeles, CA; [2]Georgetown Univ., Washington, DC; [3]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Background:
Post-mortem and imaging studies provide converging evidence that frontal lobe myelination trajectory is dysregulated in schizophrenia and suggest that, early in treatment, antipsychotic medications increase myelination and specifically intracortical myelin (ICM). We used magnetic resonance imaging (MRI) to examine whether in schizophrenia, the ICM trajectory is dysregulated compared to healthy control subjects, altered by treatment with oral antipsychotics, and associated with cognitive performance.
Methods:
Frontal lobe ICM volume was estimated using a novel method that combines distinct tissue contrasts provided by inversion recovery (IR) and proton density (PD) MRI images. One hundred and one subjects with schizophrenia (71 males and 30 females) were examined in conjunction with 85 healthy control subjects (57 males and 28 females). At the time of MRI acquisition the subjects with schizophrenia had lifetime oral antipsychotic medication exposures ranging from 0-333 months.
Results:
When plotted against medication exposure, the ICM trajectory of subjects with schizophrenia reached a maximum value at one year of antipsychotic treatment, significantly increasing during the first year of treatment and significantly decreasing thereafter. In subjects with schizophrenia, better cognitive processing speed and working memory were associated with greater ICM volume. ICM of subjects with schizophrenia declined with age, while ICM of healthy control subjects did not.
Conclusions:
In vivo MRI can dissect subtle brain tissue differences and could help clarify mechanisms of action of treatment interventions. In adults with schizophrenia, oral antipsychotic treatment is associated with an increase in ICM during the first year of treatment followed by a decline despite continued treatment. This ICM trajectory resembles clinically observed antipsychotic response trajectory with high rates of remission in the first year followed by progressively lower response rates. The inverted U of the ICM trajectory also resembles the typical pattern of initial antipsychotic medication adherence followed by nonadherence. Antipsychotic treatment may modify the tendency for ICM to decline in schizophrenia, possibly through inhibition of the constitutively active enzyme glycogen synthase kinase 3 (GSK3). Dopamine and serotonin receptor blockade provided by antipsychotics inhibit GSK3 and promote myelination. The results support post-mortem evidence that schizophrenia pathophysiology involves ICM deficits and suggest that correcting these deficits may be one mechanism of action of antipsychotics.

Disclosures: **T.A. Tishler:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Scientific Affairs, LLC. **G. Bartzokis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Scientific Affairs, LLC. **P.H. Lu:** None. **E.P. Raven:** None. **M. Khanoyan:** None. **C. Kirkpatrick:** None. **M. Pyle:** None. **P. Villablanca:** None. **L.L. Altshuler:** None. **J. Mintz:** None. **J. Ventura:** None. **L. Casaus:** None. **K.L. Subotnik:** B. Contracted

Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Scientific Affairs, LLC. **K.H. Nuechterlein:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Scientific Affairs, LLC. **B.M. Ellingson:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen Scientific Affairs, LLC.

Poster

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Topic: H.03. Schizophrenia

Support: USPHS grant P50-MH103222

Title: Causal relationship between the antioxidant glutathione, kynurenic acid and glutamate in rat prefrontal cortex

Authors: *H.-Q. WU, R. SCHWARCZ;
Maryland Psychiatric Res. Ctr., Baltimore, MD

Abstract: Oxidative mechanisms play key roles in brain physiology and pathology. The neuromodulatory tryptophan metabolite kynurenic acid (KYNA), which can control normal and abnormal cognitive processes in rodents (Schwarcz et al., 2012), is biosynthesized from its precursor L-kynurenine (L-KYN), both by irreversible enzymatic transamination [via kynurenine aminotransferases (KATs)] and by oxidation (Blanco-Ayala et al., 2015). Here, we tested the respective contributions of these two mechanisms by performing microdialysis in the prefrontal cortex of unanesthetized rats, focusing mainly on a possible role of the endogenous antioxidant glutathione (GSH). Extracellular levels of KYNA and glutamate - which is bi-directionally influenced by KYNA fluctuations in the brain (Pocivavsek et al., 2011) - were used as outcome measures. Applied locally by reverse microdialysis for 2 h, GSH (1 - 5 mM) caused a dose-dependent, reversible decrease in extracellular KYNA levels (to a nadir of -26% of baseline values). Reverse dialysis of the reducing agent dithiothreitol (5 mM) produced a qualitatively similar reduction in extracellular KYNA. Perfusion with 5 mM GSH also resulted in a ~2-fold, reversible increase in extracellular glutamate levels. Co-application of KYNA (100 nM) with

GSH (5 mM) effectively prevented the increase in glutamate induced by GSH alone. In separate rats, local perfusion with L-KYN (5 μM) increased extracellular KYNA levels ~4-fold and caused a modest reduction in glutamate (-30%). This L-KYN-induced KYNA elevation was partially blocked by co-perfusion of GSH (5 mM) alone and completed abolished by the combination of GSH (5 mM) and ESBA (3 mM), a selective inhibitor of the major KAT isozyme KAT II. Finally, reverse dialysis of the GSH synthesis inhibitor L-buthionine sulfoximine (5 mM; 4 h) produced a relatively slow, reversible increase in extracellular KYNA (peak: 160% of baseline levels). Taken together, these results suggest that the astrocyte-derived α7 nicotinic and NMDA receptor antagonist KYNA may constitute a mechanistic link between endogenous GSH and glutamate in the rat prefrontal cortex. As all three of these compounds have been proposed to participate in the pathophysiology of schizophrenia and other major brain diseases, careful scrutiny of this link may lead to novel insights regarding the etiology and treatment of these diseases.

Disclosures: H. Wu: None. **R. Schwarcz:** None.

Poster

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Topic: H.03. Schizophrenia

Support: NIH MH076060

NIH MH080272

Title: Characterization of cellular localization of OTX2 in human prefrontal cortex and its alterations in schizophrenia

Authors: *K. M. ATHANAS[1], M. I. ARDELT[1], S. BERRETTA[2,3], W. T.-U. WOO[1,4,3,5]; [1]Lab. for Cell. and Mol. Neuropathology, [2]Lab. of Translational Neurosci., McLean Hosp., Belmont, MA; [3]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA; [4]Dept. of Psychiatry, Beth Israel Deaconess Med. Ctr., Boston, MA; [5]Program of Neurosci., Harvard Univ., Boston, MA

Abstract: OTX2 is a homeobox transcription factor that has long been known to play a key role in many aspects of early embryonic brain development. Increasing evidence from rodent studies suggests that OTX2 may also play a key role in orchestrating the postnatal development of the cerebral cortex by regulating the postnatal maturation of the inhibitory interneurons that contain parvalbumin (PVB) and extracellular matrix structures called perineuronal nets (PNNs). More

recent research suggests that OTX2 is synthesized and secreted from the choroid plexus and is transported via the cerebral spinal fluid (CSF) to reach cortical destinations. Deficits of OTX2 may therefore contribute to disturbances of PVB neurons and PNNs and hence cortical circuitry dysfunction in schizophrenia. Our preliminary findings suggest that OTX2 is not only associated with PVB neurons and neurons that are encapsulated with PNNs but is also found in non-PVB cells, including possibly glia, and non-PNN-encapsulated cells. In this study, we will use double immuno-colocalization to characterize the specific cellular stoichiometry of OTX2 localization in the human prefrontal cortex (PFC) and its alterations in schizophrenia. We will also investigate whether OTX2 concentration in the CSF may be altered in subjects with this illness.Altogether, findings of this study will deepen our insight into the possible pathophysiological mechanisms that underlie PFC dysfunction in schizophrenia and may shed light on novel, targeted therapeutic strategies.

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Poster

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Title: Glutamatergic signaling is disrupted in the PSD of the amygdala and the nucleus accumbens in schizophrenia

Authors: J. CESARE[1], A. BANERJEE[1], S. WILLARD[1], N. BOWMAN[1], C.-G. HAHN[1], *K. BORGMANN-WINTER[2,3];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Psychiatry, Univ. Pennsylvania, Philadelphia, PA; [3]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Previously, we reported the first direct evidence for altered glutamatergic signaling, i.e., decreased GluN2 phosphorylation, which was traced to hypoactivity of Src kinases in the postmortem DLPFC of patients with schizophrenia. Here, we examine these dysregulations in the amygdala and nucleus accumbens (NA), which are implicated in negative symptoms of schizophrenia.Postmortem amygdala and NA from 10 matched pairs of schizophrenia patients and controls were fractionated for PSD enrichments. Liquid chromatography-selected reaction

monitoring mass spectrometry (LC-SRM/MS) was used to quantify 250+ proteins. The resulting protein expression was correlated with findings from a previously conducted Src kinase activity assay. Decreased Src activity and altered association of GluNs with signaling partners were observed in the NA and amygdala in schizophrenia. In proteomics analyses, the two brain regions show parallel increases in GluN2B ($p<0.05$), but differential alterations by region in mGluR2/3. Presynaptic vesicular proteins and adhesion molecules were strikingly decreased in the amygdala, but less so in the NA in schizophrenia. Src kinase activity was highly correlated with GluN1 ($r=.46, p<0.05$) and CAMKII ($r=.44, P<0.05$) in the PSD enrichments. Our results show molecular signatures for altered NMDA receptor signaling in the amygdala and NA of patients with schizophrenia. As shown in the DLPFC, both brain regions exhibit Src hypoactivity, with differing molecular underpinnings between regions. The amygdala and NA of schizophrenia cases show overall parallel changes in glutamatergic receptors, PSD signaling proteins and presynaptic vesicular molecules, pointing to glutamate release and post-synaptic receptor signaling as loci of dysregulation.

Disclosures: J. Cesare: None. **A. Banerjee:** None. **S. Willard:** None. **N. Bowman:** None. **C. Hahn:** None. **K. Borgmann-Winter:** None.

Poster

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Topic: H.03. Schizophrenia

Support: JSPS KAKENHI Grant Number 16K07002 to FO

The Science Research Promotion Fund to FO

Title: Emerging roles of Disrupted-In-Schizophrenia 1 in microglia

Authors: *F. OGAWA, A. NOMURA, K. MATSUZAKI, S. MIYAKE;
Juntendo Univ. Sch. of Med., Tokyo, Japan

Abstract: Microglia are the resident immune cells in the CNS that play crucial roles in maintaining brain homeostasis. Dysregulated microglial function has been increasingly implicated in the pathophysiology of psychiatric disorders such as schizophrenia. Among schizophrenia risk genes identified to date, *Disrupted-In-Schizophrenia 1* (*Disc1*) is considered to be one of the most convincing risk factors. Although its biological significance has been studied intensively in neurons, functional roles of Disc1 in microglia remain completely

unknown. Here, we performed a large scale co-immunoprecipitation assay using a mouse microglial cell line coupled to LC-MS/MS analysis to dissect the contribution of Disc1 to microglial function. We identified a number of novel Disc1-binding partners, some of which are likely to be expressed predominantly in microglia, suggesting microglia-specific roles of Disc1. Of particular interest are those that regulate cytoskeletal rearrangement and intracellular cytokine trafficking since microglia are highly motile and also known as a major source of inflammatory cytokines in the brain. Furthermore, live imaging of microglial cells expressing either mutant forms of Disc1 or a Disc1-siRNA was conducted and reveals that Disc1 profoundly influences motility of microglial cells. We therefore propose that Disc1 may participate directly in regulation of intracellular cytokine transport and cell motility in microglia. Impaired Disc1 function may cause dysregulation of these biological processes in microglia, which could potentially lead to increased risk for psychiatric disorders.

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Poster

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Topic: H.03. Schizophrenia

Support: NHMRC Fellowship APP1002240

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NHMRC Project Grant APP1045619

Title: Changed cortical BQCA modulation of [^{3}H]NMS binding in schizophrenia

Authors: ***B. DEAN**[1], S. HOPPER[1], J. CONN[2], E. SCARR[1];
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Abstract: The hypothesis that activation of cortical muscarinic M1 receptor (CHRM1) will improve the symptoms of schizophrenia can now be challenged using CHRM1 allosteric modulators. However, whilst allosteric modulators have been shown to change the activity of CHRMs using cloned human CHRMs and CHRM knockout mice, data on CHRM1 in human CNS is lacking. Here we show, *in vitro*, that BQCA, a positive allosteric CHRM1 modulator, brings about the expected change in affinity of the CHRM1 orthosteric site for acetylcholine in human cortex. That there was no change in [^{3}H]NMS binding to the cortex from subjects

schizophrenia, whether or not they had changed levels of CHRM1. By contrast there was a 57% reduction in responsiveness, as measured as the area within the acetylcholine shift cause by 3uM BQCA, to BQCA in the cortex of a subset of subjects with schizophrenia who have a profound loss (-75%) of CHRM1 as measured using [^{3}H]pirenzepine binding. These data suggest BQCA responsiveness is not simply governed by CHRM1 levels in the human cortex. To better understand the control of [^{3}H]pirenzepine and [^{3}H]NMS binding to human cortex we studied the impact of Zn^{2+} and Mg^{2+} which have both been shown to affect the binding sites on G-protein coupled receptors. We showed total [^{3}H]pirenzepine and [^{3}H]NMS binding was reduced by Zn^{2+}, acetylcholine displacement of [^{3}H]NMS binding was enhanced by Mg^{2+} and Zn^{2+}, acetylcholine displacement of [^{3}H]pirenzepine was reduced by Mg^{2+} and enhanced by Zn^{2+} whilst BQCA effects on [^{3}H]NMS, but not [^{3}H]pirenzepine, binding was enhanced by Mg^{2+} and Zn^{2+}. These data suggest the orthosteric and allosteric sites on CHRMs respond differently to divalent cations and changes in cortical micro-environments may contribute to drug binding to CHRM1 in the human cortex.

Disclosures: B. Dean: None. **S. Hopper:** None. **J. Conn:** 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; Research funding from Bristol Myers-Squibb, Johnson and Johnson, AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents that protect different classes of M1 PAMs. F. Consulting Fees (e.g., advisory boards); Consultant Pfizer. **E. Scarr:** None.

Poster

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Topic: H.03. Schizophrenia

Support: 1R01MH105608-01A1

Title: Neurons expressing parvalbumin in the thalamic reticular nucleus and their potential role in the pathophysiology of schizophrenia

Authors: *S. A. BUKHARI[1], H. PANTAZOPOULOS[1,2], S. BERRETTA[1,2,3];
[1]Translational Neurosci. Laboratory, Basic Neurosci. Div., McLean Hosp., Belmont, MA; [2]Dept. of Psychiatry, [3]Program of Neurosci., Harvard Med. Sch., Boston, MA

Abstract: Background: Growing evidence from imaging studies points to a disruption of thalamo-cortical connectivity as a critical element of the pathophysiology of schizophrenia (SZ) and, potentially, bipolar disorder (BD). This study focuses on the Thalamic Reticular Nucleus (TRN), a nucleus critically involved in modulating cortico-thalamic-cortical circuits. The TRN has been postulated to enhance cognitive and emotionally relevant stimuli while suppressing irrelevant stimuli - functions that resonate with symptoms of these disorders, such as disruption of sensory gating and emotion processing. We hypothesize that the TRN may be impacted in the pathophysiology of SZ and BD. As a fist step toward testing this hypothesis we measured numbers of neurons expressing parvalbumin (PVB), a predominant TRN neuronal population, in subjects with these disorders. **Methods:** Serial sections containing the TRN from 15 SZ patients, 15 BD patients, and 20 control subjects were immunostained using antibodies raised against PVB. Computer-assisted quantitative light microscopy was used to estimate total numbers and numerical densities of PVB-immunoreactive (IR) neurons as well as TRN volume. Linear regression models were used to test the effect of diagnosis as well as the effects of potentially confounding variables, such as age, gender, exposure to pharmacological agents and substance abuse. **Results:** In subjects with SZ, we detected significant reductions of PVB-IR numerical density ($p=0.0001$) and total numbers ($p=0.0001$) as compared to healthy controls. Numbers of PVB-IR neurons significantly and positively correlated to antipsychotic exposure within the last 6 months of life. Similarly, significant decreases of total numbers ($p=0.001$) and numerical density ($p=0.001$) were found in subjects with BD. **Conclusions:** Decreased numbers of PVB-IR neurons were observed in the TRN in both SZ and BD. Results from these studies are interpreted in the context of parallel decreases of perineuronal nets in the TRN of both these disorders, detected in a parallel study. Together these findings point to an overall reduction of PVB neurons. Deficits of PVB-IR neurons within the TRN may contribute to a dysregulation of thalamo-cortical circuitry and, in turn, to disruption of emotional and cognitive processing in SZ and BD.

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Poster

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Topic: H.03. Schizophrenia

Support: NIH Grant R01MH094358

Title: Modeling heterochromatin: Method for determining epigenetic effects of antipsychotics at immune promoters

Authors: *B. M. FEINER[1], J. K. MELBOURNE[1], K. A. CHASE[2], R. P. SHARMA[1];
[1]Psychiatry, UIC Psychiatry, Chicago, IL; [2]Psychiatry, Univ. of California, La Jolla, CA

Abstract: Background: Schizophrenia is a debilitating mental illness affecting about 2.4 million American adults. Dysregulation of immune function, notably elevated levels of certain cytokines, is a perennial finding in the schizophrenia literature. That said, the majority of individuals diagnosed with schizophrenia are prescribed one or more antipsychotics. These drugs classically function through dopamine D2 receptor antagonism, increasing intracellular levels of cAMP and thus PKA activity, which affects numerous kinase cascades, some of which elicit epigenetically permissive changes in the nucleus. In this study, we looked to determine what effect antipsychotic treatment might have on immune response gene promoters that can be reliably heterochromatinized through chronic treatment with the bacterial endotoxin lipopolysaccharide (LPS).
Methods: Human SW872 cells which express both D2 and the LPS receptor TLR4 were cultured in the presence of LPS, Risperidone, or both for 24 hours. Cells were harvested and underwent chromatin immunoprecipitation for a transcriptionally permissive euchromatin mark (phospho-H3S10) and transcriptionally repressive heterochromatin marks (HP1γ, H3K9me2). Expression levels of epigenetic modifications at the promoters of IL6, TNFα, PPARγ, and IL1β of were determined via qPCR, with GAPDH and ZNF333 promoters as controls. Values are expressed as pulldown relative to input control. To determine functional significance, mRNA levels were also evaluated using qPCR, with values normalized to GAPDH and β-actin.
Results: LPS alone significantly increased heterochromatin marks after 24 hours, which resulted in significantly decreased mRNA levels. Risperidone alone significantly increased phospho-H3S10 levels from vehicle, while also significantly increasing phospho-H3S10 levels from LPS treatment levels when cells were treated with LPS and Risperidone concurrently. HP1γ and H3K9me2 levels significantly increased with LPS treatment, but were reduced to at or near vehicle levels with concurrent Risperidone treatment. Functionally, Risperidone was found to have a significant normalizing effect on mRNA levels of IL6, TNFα, PPARγ, and IL1β when cells were treated with both LPS and Risperidone, resulting in mRNA expression at or near vehicle levels.
Discussion: This platform provides a streamlined method to examine the downstream epigenetic effects of different antipsychotic medications. The fact that heterochromatin can reliably be assembled on specific gene promoters makes for an intriguing methodological jumping off point in not only the epigenetic effects of antipsychotics, but other compounds as well.

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Poster

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Title: Inhibition of the schizophrenia associated microRNA miR-137 disrupts neuregulin-induced protein synthesis

Authors: *K. THOMAS[1], B. ANDERSON[1], N. SHAH[1], Q. GU[1], G. J. BASSELL[1,2];
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Abstract: Schizophrenia is a debilitating neurodevelopmental disorder with no cure and complex underlying genetics. Recent genome wide association studies have repeatedly found that genetic variants near and within *MIR137*, which encodes the microRNA (miRNA) miR-137, are enriched within schizophrenia patient populations. miRNAs are small noncoding RNAs that regulate mRNA translation through direct complementary base pairing to their mRNA targets. Many predicted miR-137 targets have previously been associated with schizophrenia through independent genetic studies, yet relatively few have been experimentally validated. Among these predicted targets are components of the glutamate and neuregulin (Nrg) signaling pathways, both of which may be dysregulated in schizophrenia patients. While both pathways have previously been shown to regulate neuronal development and protein synthesis, their relationships to miR-137 remain largely unexplored. In the present study, we examine the molecular relationship between miR-137, the glutamate receptor subunit GluA1, and signaling ligand neuregulin-1α (Nrg1). We find that acute stimulation with a soluble form of Nrg1 increases GluA1 levels and phosphorylation of the ribosomal protein S6 in dendrites of primary hippocampal neurons when measured by quantitative immunofluorescence. When endogenous miR-137 is inhibited, however, both responses are ablated. Puromycin labeling and proximity ligation assay experiments show that Nrg1 stimulates the synthesis of proteins including GluA1 and that this stimulation is also miR-137 dependent. Western blot analysis indicates that manipulation of miR-137 may disrupt the levels of several key proteins that lie downstream of Nrg1, providing a mechanism by which altered miR-137 activity may disrupt Nrg1 signal transduction. Together these data elucidate a novel role for miR-137 in the regulation of Nrg1-dependent mRNA translation in the dendrites of hippocampal neurons, and link miR-137, GluA1, and Nrg1 together in a novel schizophrenia associated translational control pathway.

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Poster

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Topic: H.03. Schizophrenia

Support: (NIH) R01MH094358

Title: Activated pSTAT1 levels as a biologically relevant immune signal in schizophrenia

Authors: ***J. K. MELBOURNE**[1], B. FEINER[1], C. ROSEN[1], K. A. CHASE[1,2], R. P. SHARMA[1,3];
[1]Psychiatry, Univ. of Illinois At Chicago, Chicago, IL; [2]Psychiatry, Univ. of California, San Diego, CA; [3]Jesse Brown Veterans Affairs Med. Ctr., Chicago, IL

Abstract: Background: A subclinical immunoreactive state, broadly characterized by elevated levels of serum/plasma cytokines as well as several parameters of immune cellular functioning, has been widely reported in subjects with schizophrenia. Activation of STAT1 is directly downstream of cytokine receptors that relay the signal from specific pro-inflammatory cytokines (IFNγ, IL-6, IL-2, and IL-10) shown to be dysregulated in schizophrenia, as well as a number of additional circulating molecules. If the increased cytokine levels repeatedly observed in schizophrenia have biological consequences, then the measurement of pSTAT1 is a logical step forwards. **Methods**: Peripheral blood mononuclear cells (PBMCs) from controls (n=13) and participants with schizophrenia (n=22) were extracted using a Ficoll density gradient. Participants with schizophrenia were diagnosed using the SCID, clinical symptomology was measured using the Positive and Negative Syndrome Scale (PANSS), and cognitive functioning was measured using the MATRICS Consensus Cognitive Battery. Levels of activated STAT1 (Y701; phosphorylated STAT1; pSTAT1) were measured by ELISA in nuclear extracts from PBMCs. **Results:** There was a significant bimodal distribution in the sample, with a subgroup of participants with schizophrenia expressing significantly greater levels of activated pSTAT1 than the remainder of participants. In this schizophrenia subgroup, levels of pSTAT1 were 68% higher than the control group, and 62% higher than the remainder of the participants with schizophrenia. Furthermore, this subsample of participants manifested significantly poorer cognitive performance on several measures of the MATRICS. **Discussion:** pSTAT1 levels may provide a measure of the biological relevance of widely reported elevations in levels of cytokines in individuals with schizophrenia over the past several decades. Activation of kinase cascades

can be used to partition or disassemble the composite immune signal in living patients with schizophrenia. As PBMCs are a heterogeneous cell population, using flow cytometry to measure pSTAT1 levels will enable us to determine which cell types exhibit high levels of activated STAT1 and give deeper insight into the potential consequences of altered cell signaling in schizophrenia.
Keywords: STAT1, peripheral blood mononuclear cells (PBMCs), immune, inflammation, schizophrenia, phosphorylation, cytokine

Disclosures: J.K. Melbourne: None. **B. Feiner:** None. **C. Rosen:** None. **K.A. Chase:** None. **R.P. Sharma:** None.

Poster

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Topic: H.03. Schizophrenia

Title: Effects of selective attention on gamma activity in auditory steady-state response (ASSR): an EEG study.

Authors: *A. TOYOMAKI[1], A. MIYAZAKI[2], N. HASHIMOTO[2], I. KUSUMI[2];
[1]Hokkaido univ, Sapporo, Japan; [2]Hokkaido Univ., Sapporo, Japan

Abstract: In recent years, gamma synchronization during auditory steady-state response (ASSR) paradigm has been used in the field of schizophrenia research. Most studies reported significant reduction of gamma activity in patients with schizophrenia. Some studies proposed that abnormality of gamma synchronization is derived from the dysfunction of cortical pyramidal neuron and GABAergic inter-neuron. However little attention has been given to effect of attentional modulation on gamma activity. Most patients with schizophrenia have deficits of sustained attention and thus this might affect reduction of gamma synchronization. Previous studies using ASSR paradigm have been inconclusive for attentional modulation on magnitude of gamma rhythm. Our goal was to investigate effect of selective attention on gamma synchronization in ASSR paradigm. We used auditory stimulus which was intermissive at 40 Hz and had a 500 ms duration. This tone was presented with an average 1.5 s ISI. This stimulus was regarded as standard stimulus. On the other hand, infrequently same tone was presented with 200 ms ISI. This stimulus was regarded as standard stimulus and then participants should press a button. This oddball like task allowed that participants allocate different attentional resource to physically same stimulus. We thought that target stimulus demand much selective attention than standard stimulus. Thirty-four normal controls participated in this EEG study. We conducted on

time frequency analysis to standard stimulus and target one respectively and compared for EEG synchronization. The results demonstrated that there were no differences between these stimuli. On the other hand, delta, theta and alpha band synchronization were augmented in target stimulus. Attentional modulation in present study does not affect gamma band activity. We concluded that evoked gamma activity is derived from exogenous sensory process.

Disclosures: A. Toyomaki: None. **A. Miyazaki:** None. **N. Hashimoto:** None. **I. Kusumi:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 466.15/MMM26

Topic: H.03. Schizophrenia

Support: Brain and Behavior Research Foundation

Title: The acute and chronic effects of ketamine on cross-frequency couplings and alterations in locomotive speed in the rat hippocampus: Implications for translational models of schizophrenia.

Authors: *T. I. MICHAELS, L. L. LONG, I. H. STEVENSON, J. J. CHROBAK, C.-M. A. CHEN;
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Abstract: Disrupted neuronal oscillations have been identified as a potentially important biomarker for the perceptual and cognitive symptoms of schizophrenia. Emerging evidences suggest that interactions between different frequency bands, cross-frequency coupling (CFC), serve an important role in integrating sensory and cognitive information and may contribute to the pathophysiology of schizophrenia. Animal studies have utilized ketamine as a pharmacological model of disrupted neuronal oscillations, but few studies have examined its effects on CFC. While ketamine is known to increase locomotive speed in animals, the relation between speed and CFC is not well understood. In the present study, we investigated the acute and chronic effects (14-day period) of ketamine (30 mg/kg i.p. each day) versus saline on alterations in the coupling of theta (6-12 Hz) phase and gamma (50-100 Hz) amplitude in the CA1 region of the rat hippocampus. We hypothesize that there would be both acute and chronic ketamine effects on cross-frequency coupling which would vary as a function of locomotive speed. Electrode recordings were conducted while the rats were running in a four-arm maze. Physiology data were matched by locomotive speed across animal, condition and time point in order to control for variations in speed when analyzing the CFC data. Visual inspection

confirmed an ellipse between 60-90Hz amplitude and 5-14Hz phase. The ellipse was analyzed by fitting two Gaussian distribution and analyzing changes in the position of the center frequency. Results indicate a chronic shift in the post-injection CFC for the ketamine group but not the saline group for both phase ($F(2,22)= 5.61$, $p=0.01$) and amplitude ($F(2, 22)=3.36$, $p= 0.04$). CFC strength also varied as a function of locomotive speed. Our results demonstrate that chronic ketamine administration alters the interaction of low-frequency phase and high-frequency oscillations in the rat hippocampus. These findings provide evidence that CFC may serve as an important neuronal mechanism for cognitive and perceptual processes known to be impaired in schizophrenia.

Disclosures: T.I. Michaels: None. **L.L. Long:** None. **I.H. Stevenson:** None. **J.J. Chrobak:** None. **C.A. Chen:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 466.16/MMM27

Topic: H.03. Schizophrenia

Support: R01MH090067

R01MH091130

Title: Over-expression of CNRIP in the ventral hippocampus of rodents produces a schizophrenia-like phenotype

Authors: *A. M. BOLEY[1], S. M. PEREZ[1], J. J. DONEGAN[2], D. D. AGUILAR[2], A. GIUFFRIDA[2], D. J. LODGE[2];
[1]Dept. of Pharmacol., [2]Pharmacol., UTHSCSA, San Antonio, TX

Abstract: Schizophrenia is a debilitating disease that affects up to 1% of the population with an onset of symptoms starting in late adolescence to early adulthood. This is also a period of development when many adolescents experiment with drugs and abuse cannabis. Cannabis use has been shown to increase the risk of schizophrenia in genetically susceptible individuals and can trigger psychotic symptoms. Taking the growing legalization of marijuana into consideration, it is important to understand the role of the endocannabinoid system in the pathology of schizophrenia. We have previously shown that aberrant dopamine system function is secondary to a hyperactive hippocampus driving the multisynaptic circuit. In addition, it has been postulated that there are alterations in the endocannabinoid system that contribute to the

pathogenesis of schizophrenia, known as the cannabinoid hypothesis of schizophrenia. In this experiment, we examined the role of cannabinoid receptor interacting protein (CNRIP) in the pathology of schizophrenia. Sprague Dawley rats were bilaterally injected with lentivirus expressing CNRIP1 in the ventral hippocampus to increase expression. We then examined behavioral correlates of symptoms associated with schizophrenia followed by electrophysiology to determine the effects on the dopamine system. We found that overexpression of CNRIP led to impairments in social interaction and a deficit in latent inhibition similar to what is observed in patients and rodent models of schizophrenia. Furthermore, there was a significant increase in VTA dopamine neuron population activity in rats overexpressing CNRIP. These data provide evidence that CNRIP may be involved in the pathophysiology of schizophrenia, as it was sufficient to produce various physiological and behavioral correlates of the disease.

Disclosures: **A.M. Boley:** None. **S.M. Perez:** None. **J.J. Donegan:** None. **D.D. Aguilar:** None. **A. Giuffrida:** None. **D.J. Lodge:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 466.17/MMM28

Topic: H.03. Schizophrenia

Title: Links between auditory hallucination and regional functional dysconnectivity in schizophrenia

Authors: *D. K. SHUKLA, J. CHIAPPELLI, P. KOCHUNOV, L. M. ROWLAND, L. HONG; Psychiatry, Univ. of Maryland, Baltimore, MD

Abstract: Schizophrenia (SCZ) is a severe mental illness characterized by positive and negative symptoms as well as behavioral, motor and mood disturbances. Auditory hallucinations are one of the most debilitating symptoms of SCZ. Resting-state functional magnetic resonance imaging (rsfMRI) studies have suggested various brain network abnormalities associated with auditory hallucinations in SCZ patients. In this study, we used a data driven regional homogeneity (ReHo) approach to measure the temporal homogeneity of blood oxygen level dependent (BOLD) signal for exploring regional brain concordance in patients with SCZ. Resting-state fMRI data of 96 patients with SCZ and 90 healthy control (HC) participants were acquired. Motion-corrected time series for each participant were detrended. Kendall's coefficient of concordance (KCC) was used to measure correlation between the time series of each voxel and those of its 27 nearest neighbors. Furthermore, KCC was correlated with perception auditory state and trait scores. Multiple comparisons correction was performed to a significant level of $p < 0.005$ with a

minimum cluster size of 79 voxels. ReHo results showed reduced KCC in the SCZ group compared to the HC group in the default mode regions such as bilateral medial prefrontal cortex, posterior cingulate cortex, precuneus, and middle temporal lobe. Reduced KCC was also found in other regions associated with auditory-verbal hallucinations in the anterior cingulate, inferior frontal gyrus (including Broca's area) and superior and middle temporal gyri (including Wernicke's area) bilaterally. Regression analysis showed that KCC was negatively correlated with perception auditory state score in the left inferior frontal gyrus (including Broca's area), left middle frontal gyrus, right precentral gyrus, right lingual gyrus, bilateral posterior cingulate and cuneus. Perception auditory trait score was also negatively correlated with KCC in the right posterior cingulate and bilateral cuneus. Our results point toward a reduced connectivity between frontal and temporal language areas in SCZ patients, which may be driven by auditory hallucinations. Reduced connectivity in the default mode regions may imply sociocommunicative impairments in SCZ. Our results add to limited rsfMRI findings of abnormal local functional connectivity in SCZ patients, which could reflect impaired local functional differentiation. Our study served to explore the ReHo approach in SCZ, which is complementary to the conventional model-driven activation approach. The ReHo approach may also help subsequent inferential methods to reduce type 1 error due to heterogeneity of fMRI timecourses.

Disclosures: D.K. Shukla: None. **J. Chiappelli:** None. **P. Kochunov:** None. **L.M. Rowland:** None. **L. Hong:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Program#/Poster#: 466.18/MMM29

Topic: H.03. Schizophrenia

Support: Grant-in-Aid for Scientific Research(A)(25242078) from MEXT of JAPAN

Title: Three-dimensional analysis of dendritic spines and mitochondria in dentate gyrus granule cells in Schnurri-2 knockout mice, an animal model for schizophrenia

Authors: *A. NAKAO[1], K. TAKAO[2,4], K. OHIRA[5], N. MIYAZAKI[3], K. MURATA[3], T. MIYAKAWA[1,2];
[1]Fujita Hlth. Univ., Toyoake, Aichi, Japan; [2]Section of Behavior Patterns, Ctr. for Genet. Analysis of Behavior,, [3]Natl. Inst. for Physiological Sci., Okazaki, Japan; [4]Div. of Animal Resources and Development, Life Sci. Res. Ctr., Univ. of Toyama, Toyama, Japan; [5]Dept. of Food Sci. and Nutr., Mukogawa Women’s Univ., Nishinomiya, Japan

Abstract: Accumulating evidence suggests that morphological changes of subcellular-scale structures such as dendritic spine and mitochondria may be involved in the pathogenesis/pathophysiology of schizophrenia. Previously, we have proposed mice lacking Schnurri-2 (Shn2), an MHC enhancer binding protein, as a schizophrenia model with mild chronic inflammation. In the mutants, there are decreases in the expression level of PSD95, synaptic marker, and increases in C1q family genes (C1qa, C1qc and C1ql2), which are considered to mediate synapse elimination during the postnatal development. In the present study, we analyzed three-dimensional morphological changes in dendritic spines and mitochondria in dentate gyrus granule cells in Shn2 knockout (KO) mice by serial block-face scanning electron microscopy. The mutants showed about 13% increase in spine length, and about 25% increase in spine neck length. There were no significant differences between Shn2 KO and wild-type mice in their spine density, volume of spine, spine head length, or spine head diameter. The mutants exhibited decreased number of constricted mitochondria, suggesting that a balance between mitochondrial fusion and fission is compromised in Shn2 KO mice. Additionally, there were significant decreases in nuclear volume in the mutants. These morphological changes may be associated with functional impairments of those subcellular-scale structures, and represent potential endophenotype of schizophrenia.

Disclosures: **A. Nakao:** None. **K. Takao:** None. **K. Ohira:** None. **N. Miyazaki:** None. **K. Murata:** None. **T. Miyakawa:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 466.19/MMM30

Topic: H.03. Schizophrenia

Support: NIH Intramural Research Program

Title: Dysbindin regulates mitochondrial fission in hippocampal excitatory neurons through the Dynamin-Like-Protein DLP1

Authors: *J. ZHAO, Z. LI;
NIMH/NIH, Bethesda, MD

Abstract: Background: Mitochondria have several functions, such as the production of energy and reactive oxygen species (ROS) and calcium buffering, which are essential for neuronal activity and connectivity. Mitochondria form networks in the cell. The mitochondrial network is dynamically regulated by mitochondrial fission and fusion, which are required for the biogenesis

and quality control of mitochondria. Mitochondria dynamics is controlled by mitochondrial fission and fusion proteins, such as MFN-1, DLP1, FIS-1 and so on. Earlier reports have implicated a possible linkage between mitochondrial dysfunction and schizophrenia. However, how schizophrenia risk genes regulate mitochondrial functions and mitochondrial network have not been investigated. Result: In our study, we investigated the role of the human schizophrenia susceptibility gene *DTNBP1*, encoding dysbindin protein, in mitochondria dynamics. We found that a lack of dysbindin in excitatory neurons results in shorter mitochondria with lower mitochondrial membrane potentials, and overexpression of dysbindin significantly changed the subcellular localization of DLP1 and acvitity-induced mitochondrial fission. With STED imaging, we found that dysbindin protein is co-localized with DLP1 on mitochondria. Furthermore, we found that mitochondria fission increases during gamma oscillation in hippocampal neurons, and blockers of mitochondrial fission reduces gamma oscillation. In dysbindin null mutant mice, hippocampal gamma oscillation significantly decreased and it can be partially rescued by dysbindin overexpression. Conclusion: Our results indicate that dysbindin regulates mitochondrial dynamics and function through DLP1, mitochondrial fission is required for gamma oscillation, and therefore dysbindin deficiency may contribute to impaired neural network activity in schizophrenia by causing mitochondrial defects.

Disclosures: **J. Zhao:** A. Employment/Salary (full or part-time): NIMH/NIH. **Z. Li:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

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Topic: H.03. Schizophrenia

Support: NIH R01MH085666

Title: GSK3β modulates NR2A subunit expression by regulating β-catenin abundance in the prefrontal cortex

Authors: *S. MONACO[1], W. GAO, 19107[2];
[1]Drexel Univ. Col. of Med., Philadelphia, PA; [2]Drexel Univ., Philadelphia, PA

Abstract: Cognition, more specifically working memory, is a fundamental process mandatory in order to successfully navigate through a persistently dynamic environment. The ability to perceive, filter, prioritize, update and ultimately act appropriately to incoming stimuli requires efficient prefrontal cortex-dependent processing, with both NMDA receptors (NMDARs) and GABAergic interneurons playing a major role. NMDA receptors are considered the cellular

entity associated with learning, memory, as well as higher-order cognitive processes including executive functioning. Additionally, GABAergic interneurons in the prefrontal cortex (PFC) regulate working memory, attention, and cognitive flexibility. Correspondingly, disruption of both NMDARs and GABAergic signaling is strongly implicated in several disorders that are characterized by cognitive impairments, particularly schizophrenia. However, what remains to be known is how NMDARs on GABAergic interneurons are regulated and how this affects physiological function. We predict GSK3β is an upstream linking factor among NMDARs, GABAergic signaling, and prefrontal-dependent cognition. Therefore disruption of this enzyme may serve as an underlying factor for common pathological phenotypes observed in schizophrenia such as NMDA hypofunctioning and reduced GABAergic signaling. Our findings demonstrate that lithium treatment in mature primary prefrontal neuronal cultures, significantly increased levels of phosphorylated GSK3βser9 and decreased β-catenin33/37/41 phosphorylation, demonstrating GSK3β inhibition and reduced β-catenin degradation. Total β-catenin and NR2A subunit levels concurrently increased following lithium treatment, suggesting that GSK3β inhibition enhanced β-catenin availability and thereby upregulated NR2A expression. We found similar changes in vivo. Our data suggests that GSK3β regulates NR2A-specific expression via β-catenin availability, which can have important implications for NMDAR expression on GABAergic interneurons.

Disclosures: S. Monaco: None. **W. Gao:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Program#/Poster#: 466.21/MMM32

Topic: H.03. Schizophrenia

Support: NHMRC #APP1067137

Title: Dysregulation of schizophrenia-associated genes by elevation of microRNA biogenesis machinery

Authors: *M. GEAGHAN[1,2], M. CAIRNS[1,2,3], A. BRICHTA[1,2];
[1]Univ. of Newcastle, Australia, Callaghan, Australia; [2]Priority Res. Ctr. for Translational Neurosci. and Mental Hlth., Hunter Med. Res. Inst., New Lambton, Australia; [3]Schizophrenia Res. Institue, Sydney, Australia

Abstract: MicroRNAs (miRNA) are short, ~22 nucleotide-long strands of RNA that are vital post-transcriptional regulators of gene expression. They are generated through several processing

steps by various proteins, including the nuclear microprocessor components DGCR8 and DROSHA, and the cytoplasmic RNase III DICER. MiRNAs are a significant regulatory mechanism within the cell, with a single miRNA able to target and silence hundreds of genes, making them particularly interesting in the context of polygenic disorders such as schizophrenia (SZ). Previous studies have shown an upregulation of the miRNA biogenesis genes *DGCR8*, *DROSHA*, and *DICER* in both the dorsolateral prefrontal cortex and superior temporal gyrus of people with SZ, suggesting a role for elevated miRNA production in the pathophysiology of this disorder. The aim of the current study was to identify the molecular consequences of elevated miRNA biogenesis by overexpressing either *DGCR8* or *DICER* in differentiated, neuron-like SH-SY5Y cells *in vitro*. RNA was extracted from these cells and analysed by mRNA and miRNA sequencing, as well as qPCR. Sequencing results were passed through pathway analyses to identify common themes amongst dysregulated genes. This predicted significant alterations to neuronal pathways such as axonal guidance signalling, glutamate signalling, and wnt/β-catenin signalling, which all have relevance to SZ. Furthermore, specific SZ-associated genes, such as ionotropic glutamate receptor subunits *GRIA2* and *GRIA3*, reelin (*RELN*), and the miR-137 host gene (*MIR137HG*) were found to be significantly differentially expressed. Additionally, following overexpression of *DICER*, several genes involved in inflammatory and immune pathways, including MX dynamin-like GTPase 1 (*MX1)* and interferon-induced protein 44 (*IFI44)*, were amongst the most highly upregulated genes. This result is particularly significant as the immune system is thought to play a significant role in the pathophysiology of SZ. Overall, the results of this study suggest that the overexpression of either *DGCR8* or *DICER* is able to influence neuronal function, including synapse formation and synaptic signalling, and adds support to the hypothesis that elevation of miRNA biogenesis machinery components may be part of the pathophysiology of SZ.

Disclosures: **M. Geaghan:** None. **M. Cairns:** None. **A. Brichta:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

Location: Halls B-H

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Program#/Poster#: 466.22/MMM33

Topic: H.03. Schizophrenia

Support: NSC101-2320-B-182-040-MY3; CMRPD170423

Title: dopamine D_3 receptor blockade rescues hyperdopamine activity-induced deficit in novel object recognition memory

Authors: *P.-K. CHANG, J.-C. CHEN;
Chang-Gung University/ Grad. Insititute of Biomed. Sci., Kwei-Shan Tao-Yuan, Taiwan

Abstract: Overeactivity of dopamine signaling is thought to underlie the pathophysiology of a number of psychiatric disorders, such as psychosis, mania and attention-deficit/hyperactivity disorder (ADHD). These mood disorders are frequently associated with cognitive deficits such as disturbances in attention processes or learning and memory, suggesting that persistent changes in dopamine signaling may alter neural plasticity and lead to disease progress. Mice with reduced DAT expression (knockdown, KD) display hyperdopaminergic phenotypes, hence serve as models for mania and ADHD. In comparison with WT mice, DAT KD mice exhibited a deficit in novel object recognition when test (NORT) was performed 24 h later. This deficit could be rescued by acute exposure to D_3 selective antagonist, FAUC365, before recognition training. In consistent with this result, D_3/DAT double mutants reverse the functional loss of NORT due to DAT knockdown. Further, we found acute methamphetamine treatment (1 mg/kg) impaired NORT in WT mice (no change in D_3KO mice), which could also be compensated by the FAUC365 pretreatment, indicating that synaptic dopamine strength may compromise object recognition capability through D_3R. Overall, the present data implicate dopamine D_3 receptors participate a functional role in object recognition. The underlying biomedical mediators are currently under investigation.

Disclosures: P. Chang: None. **J. Chen:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Program#/Poster#: 466.23/MMM34

Topic: H.03. Schizophrenia

Support: R25 MH101072

Title: Parkinsonism predicts personality traits related to genetic risk and treatment outcomes in schizophrenia

Authors: *J. MOLINA[1], M. CALVO[2], M. BALDA[3], G. GUERRERO[2], E. PADILLA[2], C. CLONINGER[4], G. DE ERAUSQUIN[5];
[1]Psychiatry, UCSD, Miami, FL; [2]Fundación de Lucha contra los Trastornos Neurológicos y Psiquiátricos en Minorías, Buenos Aires, Argentina; [3]Morsani Col. of Medicine, Univ. of South Florida, Tampa, FL; [4]Departments of Psychiatry and Genetics, Washington Univ. Sch. of Med.,

St. Louis, MO; [5]Div. of Neurosciences and Dept. of Psychiatry and Neurology, UTRGV Sch. of Med., Harlingen, TX

Abstract: Schizophrenia is a devastating neuropsychiatric condition with manifest social and interpersonal impairments. Identifying endophenotypes of schizophrenia is of critical importance and has profound implications on clinical practice. Here we propose an innovative approach to clarify the mechanism through which temperament and character deviance relates to risk for schizophrenia and predict long-term treatment outcomes. We recruited 61 antipsychotic-naïve subjects with chronic schizophrenia, 99 unaffected relatives, and 68 healthy controls from rural communities in the Central Andes. Diagnosis was ascertained with the Schedules of Clinical Assessment in Neuropsychiatry; parkinsonian motor impairment was measured with the Unified Parkinson's Disease Rating Scale; mesencephalic parenchyma was evaluated with transcranial ultrasound; and personality traits were assessed using the Temperament and Character Inventory. Ten-year outcome data was available for 24 (~40%) of the index cases. Patients with schizophrenia had higher Harm Avoidance and Self-Transcendence (ST), and lower Reward Dependence (RD), Cooperativeness (CO), and Self-Directedness (SD). Unaffected relatives had higher ST and lower CO and SD. Parkinsonism reliably predicted RD, CO, and SD after correcting for age and sex. The average duration of untreated psychosis (DUP) was greater than 5 years. Further, SD was anticorrelated with DUP and antipsychotic dosing at follow-up. Baseline DUP was related to antipsychotic dose years. Further, multidimensional personality profiles (i.e., '*explosive/borderline'*, '*methodical/obsessive'*, and '*disorganized/schizotypal'*) were associated with increased risk of schizophrenia. In summary, parkinsonism predicts core personality features and treatment outcomes in schizophrenia. Our study suggests that RD, CO, and SD are endophenotypes of the disease that may, in part, be mediated by dopaminergic function. Further, baseline SD may serve as an important determinant of treatment course and outcome.

Disclosures: J. Molina: None. **M. Calvo:** None. **M. Balda:** None. **G. Guerrero:** None. **E. Padilla:** None. **C. Cloninger:** None. **G. de Erausquin:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Program#/Poster#: 466.24/MMM35

Topic: H.03. Schizophrenia

Support: DFG (CIN to A.L. & HY.W.)

Graduate Training Centre of Neuroscience

Title: Delusions of influence correlate with reduced temporal binding in patients with schizophrenia

Authors: M. J. ROTH[1,2], M. J. BUEHNER[3], K. HESSE[4], D. WILDGRUBER[4], H. WONG[5], *A. LINDNER[1];
[1]Hertie Inst., Tuebingen, Germany; [2]Intl. Max Planck Res. Sch. for Cognitive and Systems Neurosci., Tuebingen, Germany; [3]Sch. of Psychology, Cardiff Univ., Cardiff, United Kingdom; [4]Dept Psychiatry and Psychotherapy, Univ. of Tuebingen, Tuebingen, Germany; [5]Werner Reichardt Ctr. for Integrative Neurosci., Tuebingen, Germany

Abstract: Delusions of influence (DoI) are a first-rank symptom and hallmark of schizophrenia. Patients suffering from DoI lack the feeling of control over their thoughts and actions. Here we tested whether such disturbed sense of agency could be captured with a temporal binding paradigm. Temporal binding describes the fact that intentional causal actions (e.g. a key press) and the sensory events triggered by them (e.g. a delayed LED flash) are bound together in subjective time: The sensory outcome of an intentional action is perceived as happening earlier in time than an identical sensory event preceded by a non-intentional signal. Such temporal binding is also referred to as "intentional binding" and is considered an implicit marker of the sense of agency. Temporal binding thus should be altered in patients suffering from DoI.
We investigated temporal binding in a group of 20 patients with schizophrenia and 13 healthy controls. Participants had to predict the time of a *target LED* flash via a timed button press. The flash of the *target LED* occurred at a fixed target interval of 500ms following either (i) a participant's button press or (ii) a *signal LED* flash. Therefore, in (i) participants' actively caused the *target LED* to flash while in (ii) there was no intentional (or causal) link between the behavior of the participant and the *signal* and *target LED* flashes, which were just following one another with the same 500ms interval.
While our control group showed clear temporal binding, i.e. significantly ($p < 0.05$) earlier button presses in condition (i) as compared to (ii), this effect was absent in the group of patients with schizophrenia. Furthermore, we found a significant negative correlation between the strength of patients' DoI and the amount of binding ($r = -0.6$, $p = 0.005$): lower scores for DoI were associated with greater temporal binding. Accordingly, when we performed a median split of our patient group based on the DoI scores, we recovered a significant binding effect in the subgroup of patients exhibiting less DoI ($p < 0.05$).
Our results show that patients with schizophrenia suffering from DoI exhibit altered temporal binding. Furthermore, we could demonstrate that reductions in temporal binding correlate with the strength of DoI. These results are paralleled by our earlier findings in which we show that patients with DoI are impaired in predicting the sensory consequences of their actions due to imprecise forward models. Such imprecise sensory predictions might likewise underlie altered temporal binding.

Disclosures: **M.J. Roth:** None. **M.J. Buehner:** None. **K. Hesse:** None. **D. Wildgruber:** None. **H. Wong:** None. **A. Lindner:** None.

Poster

466. Schizophrenia: Biochemistry and Neuropathology

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 466.25/MMM36

Topic: H.03. Schizophrenia

Support: R01 MH094445-06

Title: Decreased lactate dehydrogenase activity and abnormal expression of lactate shuttle transporters in schizophrenia

Authors: *C. R. SULLIVAN[1], K. CLICK[2], R. KOENE[2], A. RAMSEY[3], C. MIELNIK[3], R. MCCULLUMSMITH[2];
[1]Psychiatry, [2]Univ. of Cincinnati, Cincinnati, OH; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: Schizophrenia is a devastating illness that affects over 2 million people in the U.S. and costs society billions of dollars annually. Patients with schizophrenia experience a wide range of psychotic symptoms, as well as cognitive deficits and profound negative symptoms that are often treatment resistant. Synaptic transmission relies heavily on a net flow of energetic molecules from astrocytes to neurons, such as the astrocyte-neuron lactate shuttle. Glycogen rich glial cells rapidly synthesize pyruvate from glucose, convert pyruvate to lactate via lactate dehydrogenase (LDH), and transport lactate via monocarboxylate transporters (MCTs) to neurons for energetic use. Working memory performance and long term memory formation in rodents are impaired following disruption of this lactate shuttle pathway. Schizophrenia displays a number of abnormalities associated with glucose metabolism and the lactate shuttle, suggesting energy storage and usage deficits in the brain.This suggests lactate metabolism and neurotransmission are tightly coupled to cognitive function, and these pathways could be important pathophysiological substrates in schizophrenia. We hypothesize that the lactate shuttle pathway is differentially regulated in schizophrenia.
In schizophrenia, LDH activity was significantly decreased in the dorsolateral prefrontal cortex (n=16) when compared to control subjects (n=16). We assessed lactate concentrations in the DLPFC of schizophrenia (n=18) and control subjects (n=19) and found no change in lactate levels. Additionally, in an animal model of schizophrenia, the NR1 knockdown mouse model, we see substantial decreases in MCT4 (63%) and glucose 3 transporter (60%) transcripts in the frontal cortex.
Decreases in expression and activity of key lactate shuttle targets could diminish the capacity of astrocytes to supply neurons with energetic substrates. In conclusion, the lactate shuttle is altered in schizophrenia possibly contributing to abnormal neuronal transmission.

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Poster

467. Bioinformatics

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Program#/Poster#: 467.01/MMM37

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant NS016686

Title: Systematizing the construction of connectomes using an interactive Excel-based platform to facilitate data entry and collation.

Authors: *J. D. HAHN, L. W. SWANSON;
Biol. Sci., USC, Los Angeles, CA

Abstract: Recent investigations of deep-brain neural connectivity have underscored the magnitude of the complexity of the brain's neural circuits [1]. Approaches that seek to make this complexity more tractable therefore offer a route to increasing our understanding of brain structural organization, which is foundational to gaining new insights into diseases that affect the brain and nervous system. Three hierarchical (nested) levels of scale for brain connectivity may be defined [2]: 1) a top macroscale level, which includes connections between histologically defined gray-matter regions; 2) a middle mesoscale level, which includes connections between defined neuron types (defined according to specified criteria such as connections, shape, location, and gene-expression patterns); 3) a bottom microscale level, which includes the specific connections of individual neurons. Several recent high-throughput efforts seek to construct brain-wide connectomes at all three levels. While these efforts generate vast amounts of data, a potential weakness lies in the accuracy with which the data generated is analyzed and represented. This is a particular concern with automated or semi-automated methods that do not yet offer the level of critical and careful analysis that expert collators can provide. With this in mind, a different approach is to mine the existing substantial body of literature on brain connections with an expert-led collation effort. The value of this approach was demonstrated recently by construction of the first comprehensive macroscale association connectome for the cerebral cortex of the rat [3]. To streamline and fast-track the process of data entry and analysis with this approach, we have developed an interactive, highly structured Excel workbook template. The current version is designed for macroscale data entry; it may also form the basis of future versions suited to entry of mesoscale, or microscale data. The interactive template is supported by a guide and rigorous workflow, to facilitate training and participation of additional expert collators (especially student researchers) to support these efforts.
References: 1. *Hahn, J.D. & Swanson, L.W. (2015) Connections of the juxtaventromedial region of the lateral hypothalamic area in the male rat. Front Syst Neurosci. 9(66).* 2. *Swanson, L.W. & Bota, M. (2010) Foundational model of structural connectivity in the nervous system with a*

schema for wiring diagrams, connectome, and basic plan architecture. PNAS. 107(48): 20610-20617. 3. *Bota, M., Sporns, O. & Swanson, L.W. (2015) Architecture of the cerebral cortical association connectome underlying cognition. PNAS, 2015. 112(16): E2093-101.*

Disclosures: J.D. Hahn: None. **L.W. Swanson:** None.

Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 467.02/MMM38

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH grant NHGRI HG000330

NIH grant NICHD HD062499

Title: 'What does this gene do': Data presentation in Mouse Genome Informatics for the scientific community including neuroscientists

Authors: *L. NI, &. ON BEHALF OF MOUSE GENOME INFORMATICS GROUP; Mouse Genome Informatics, The Jackson Lab., Bar Harbor, ME

Abstract: The Model Organism Databases (MODs) have a strong history of i) gathering relevant data from the biomedical literature, and from data loads from other sources, ii) strenuously integrating that data for heterogeneous data types, and iii) providing that data to the scientific community in a variety of forms including web interfaces, APIs, dataMines, ftp files, and more. The Mouse Genome Informatics (MGI) project (www.informatics.jax.org), one such MOD, is the community resource for the laboratory mouse, a premier model for the study of genetic and genomic systems relevant to human biology and disease. MGI data includes data from over 220,000 publications for almost 23,000 protein-coding genes. These data include information about 46,000 mutant alleles with over 11,000 genes with mutant alleles in mice. There are over 14,000 genes with expression results, and over 24,000 genes with GO annotations. In addition, MGI captures homology data especially data about human orthologs (>17,000) and mouse models for human diseases (>4,800).
Recently, MGI completed a comprehensive revision of data summation and presentation to provide comprehensive yet interpretable overviews of information available for mouse genes. MGI Gene Detail pages have always provided links to all the information we have on the mouse gene. With this release, the Gene Detail pages display more information and provide more ways to view subsets of data and access details. New graphical displays provide a synopsis of a gene's

functions, where it is expressed and the phenotypes of mutant alleles. Particular emphasis is on the homology between human genes and diseases, with details about mouse models for these diseases. Links to curated Wikipedia pages for the human genes provide textual summation of available data
Matrix grids that highlight the highest level categories of gene function, phenotype, and expression data (sometimes referred to as 'slims') represent carefully considered high-level terms that may be transferable to other MODs and like resources. Each square in the grid drills down to the details of underlying experimental data.
As the volume of heterogeneous data continues to rapidly increase, the ability to provide high level summation of information about entities such as genes along with access to the deepest data available is essential for navigation of these data resources by the diverse scientific community including neuroscientists. MGI provides some models that may see general adoption by bioinformatics resources.

Disclosures: L. Ni: None. **&. On behalf of Mouse Genome Informatics Group.:** None.

Poster

467. Bioinformatics

Location: Halls B-H

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Program#/Poster#: 467.03/MMM39

Topic: I.02. Systems Biology and Bioinformatics

Support: Intramural Research Programs of the National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, and the National Eye Institute.

School of Informatics at the University of Sussex and the Dr. Mortimer and Theresa Sackler Foundation

Title: Three dimensional digital template atlas of the macaque brain.

Authors: C. REVELEY[1], A. GRUSLYS[2], F. YE[3], J. SAMAHA[1], D. GLEN[4], B. RUSS[5], Z. SAAD[4], A. SETH[1], D. A. LEOPOLD[6], *K. S. SALEEM[5];
[1]Sch. of Engin. and Informatics, Sackler Ctr. for Consciousness Science, Univ. of Sussex, Brighton BN1 9QJ, United Kingdom; [2]Dept. of Physics, Univ. of Cambridge, Cambridge CB3 0HE, United Kingdom; [3]Neurophysiol. Imaging Facility, Natl. Inst. of Mental Health, Natl. Inst. of Neurolog. Disorders and Stroke, and Natl. Eye Institute, Natl. Inst. of Hlth., Bethesda, MD; [4]Scientific and Statistical Computing Core, [5]Lab. Neuropsychology (LN), Natl. Inst. of Mental Hlth. (NIMH/NIH), Bethesda, MD; [6]Neurophysiol. Imaging Facility and Lab. Neuropsychology

(LN), Natl. Inst. of Mental Health, Natl. Inst. of Neurolog. Disorders and Stroke, and Natl. Eye Institute, Natl. Inst. of Health; Natl. Inst. of Mental Hlth. (NIMH/NIH), Bethesda, MD

Abstract: Macaque researchers need to determine the areal location from both anatomical and functional MRI scans. Using a 2D atlas for this purpose typically requires comparing an MRI dataset with labeled histological sections from one plane. The Saleem and Logothetis (2012) atlas offers three planes, with its areal boundaries initially identified in horizontal and coronal sections, and then interpolated into sagittal sections. However, what is really needed is a high quality 3D volumetric atlas that can be automatically computer-registered to the 3D anatomical or functional scan from any animal, and thus used to specify the areal designation relative to experimental locations of interest. One desirable feature of the Saleem and Logothetis atlas is, its initial registration to a high-resolution anatomical MRI scan of the same macaque subject, thus preserving the native, in vivo geometry of the brain. Here we present and validate a new 3D template atlas derived from and registered to original Saleem and Logothetis atlas. In addition to the template atlas itself, we outline a number of steps involved in the conversion and digitization of the 2D atlas into 3D template form, and its application to projects that involve anatomical, functional, or connectional imaging.

Steps:

1) The architectonic subregions of different cortical and subcortical areas from each 2D drawing of the sections (Saleem and Logothetis atlas) were digitized into a 3D volume of labels.
2) A surrogate anatomical MRI volume of better gray/white matter tissue contrast and higher spatial resolution was created by registering a high quality ex vivo MRI scan of a perfused brain to the original atlas space defined by the native geometry of the original MRI scan from the atlas.
3) The 3D volume of labels was then processed with anatomically constrained interpolation to match the surrogate MRI volume, followed by manual editing to remove any residual mismatch and artifacts.
4) Finally, the accuracy and usability of the 3D template atlas was demonstrated by applying the atlas data to a range of macaque brains of different sizes, and functional imaging data.

Key Points:

1) The atlas provides a readily usable standard for region definition while the template provides a standard reference and space.
2) This standard space allows for macaque research to be reported on a common basis across research sites and across macaques.
3) Additionally, the atlas allows for automated analysis against a set of standard region locations. Atlases and templates are available as both volumes and surfaces in standard NIFTI and GIFTI formats. It is worth noting that this 3D digital atlas can be used in different image registration and analysis softwares.

Disclosures: **C. Reveley:** None. **A. Gruslys:** None. **F. Ye:** None. **J. Samaha:** None. **D. Glen:** None. **B. Russ:** None. **Z. Saad:** None. **A. Seth:** None. **D.A. Leopold:** None. **K.S. Saleem:** None.

Poster

467. Bioinformatics

Location: Halls B-H

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Program#/Poster#: 467.04/MMM40

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH MS4EB020406

P41EBD15922

Title: Neuroimaging phenome-wide association study of mathematical disability

Authors: *S. YARED[1,2], C. GONZALEZ-ZACARIAS[1,2], F. SEPEHRBAND[1,2], L. ZHAO[1,2], K. LYNCH[1,2], I. MACPHEE[1,2], S. SALUJA[1,2], A. W. TOGA[1,2], K. A. CLARK[1,2];
[1]Lab. of Neuroimaging, USC Stevens Neuroimaging and Informatics Inst., [2]Keck Sch. of Med., USC, Los Angeles, CA

Abstract: Mathematical disability has long been thought to have a hereditary component. Yet, due to its high comorbidity with other learning difficulties such as dyslexia, it is difficult to study in isolation. A number of genome-wide association studies (GWAS) have suggested potential SNPs that may give insight as to the genetic nature of this mental handicap. Among them rs789859 and rs133885 have shown to be the most significantly correlated with math disability, although neither result has been widely replicated. rs789859 is located on the 3q29 chromosome, for which deletions have been associated with other learning difficulties including autism. It showed significance correlation (4.57×10^{-6}, n=542) to math disability in a 16-18 year-old cohort of students while controlling for reading ability. On the other hand, rs133885 was most significantly correlated to math disability in a dyslexic population (7.71×10^{-10}, n=699), with a smaller effect size found for a general population. Little has been established, however, as to the neuroanatomical correlates of this impairment. In the aforementioned study, rs133885 was associated with a decreased intra-parietal sulcus depth and right IPS volume. A number of other studies have pointed to IPS deficits associated with math disability, making that structure a plausible candidate. We conducted a neuroimaging phenome-wide association study (PheWAS) in an attempt to further examine a possible relationship between these structural abnormalities and the particular genotypes associated with mathematical disability. With this method, we are able to extract the particular neuroanatomical phenotypes that are most significantly modulated by these genetic SNPs across a database. We checked 628 subjects (294 males, 334 females) from the Philadelphia Neurodevelopmental Consortium, 8-22 years old (mean=14.77), for genotype data on our SNPs of interest, and ran their T1-weighted structural MR images through surface-based processing using FreeSurfer and volumetric processing with various co-registration tools. Extensive quality control was performed at every step, to preserve the integrity of the data. Anatomical parcellation was performed using an exhaustive set of atlases, ultimately

generating several thousand metrics per subject. After correcting for multiple comparisons, the gray matter volume of the hIP3 sub-region of the right anterior IPS was significantly modulated by the presence of rs133885 (1.55×10^{-11}), successfully replicating the previous finding.

Disclosures: **S. Yared:** None. **C. Gonzalez-Zacarias:** None. **F. Sepehrband:** None. **L. Zhao:** None. **K. Lynch:** None. **I. MacPhee:** None. **S. Saluja:** None. **A.W. Toga:** None. **K.A. Clark:** None.

Poster

467. Bioinformatics

Location: Halls B-H

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1R01HD086888-01

BCS-1441502

Title: Optimal trajectories for brain state transitions

Authors: ***S. GU**[1], R. F. BETZEL[2], M. CIESLAK[3], S. T. GRAFTON[3], F. PASQUALETTI[4], D. S. BASSETT[2];
[1]Applied Mathematics, Univ. of Pennsylvania, PHILADELPHIA, PA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]The Univ. of California, Santa Barbara, Santa Barbara, CA; [4]The Univ. of California, Riverside, Riverside, CA

Abstract: The complexity of neural dynamics stems in part from the architectural complexity of the underlying anatomy. Yet how the organization of the white matter architecture constrains the possible (or observed) brain states and how the brain alters its activity to transition among states along certain trajectories remain elusive. Here we use the term state to refer to a pattern of activity across neurons or brain regions and the term trajectory to refer to the path of the

transition among states. The question of modeling the underlying mechanism of the brain's state transition can be addressed by drawing on recent insights from the application of network control theory to neuroimaging data [1]. Prior work using these techniques has assessed the average or global role of white matter emanating from single brain regions in explaining state transitions [2]. In contrast, here we examine transitions that are elicited via the collective control of region sets [3]. This choice is motivated by our growing understanding of large-scale brain dynamics, as measured by functional magnetic resonance imaging. Specifically, we examine how the brain moves from a specified initial state to a specified target state in finite time, and we focus on examining transitions from the so-called default mode into target states of high activity in primary sensorimotor cortex. Across all state transitions, we observe that the supramarginal gyrus and the inferior parietal lobule consistently acted as efficient control hubs with less energy cost. These areas are structurally interconnected with ventral premotor complex, the key input to primary sensorimotor cortex. We further quantify the robustness of the control tasks and compare the associated energetic impact of nodes by removing them from the network. We observe that compared to healthy individuals, patients with mild traumatic brain injury (mTBI) display a higher level of robustness to removal, potentially due to greater homogeneity in network structure. Together these results suggest that network control theory may offer new insights into the mechanisms driving brain state transitions in support of behavior.

[1] P. Fabio, Zampieri, etc (2014). Controllability metrics, limitations and algorithms for complex networks. Control of Network Systems, IEEE Transactions on, 1, 40-52.
[2] Shi Gu, Fabio Pasqualetti, Danielle S. Bassett, etc, Controllability of structural brain networks, Nature Communications 6, 8414 (2015).
[3] Betzel, Richard F, Shi, Gu, Medaglia, John D, Pasqualetti, Fabio & Bassett, Danielle S (2016). Optimally controlling the human connectome: the role of network topology. arXiv preprint arXiv:1603.05261.

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Poster

467. Bioinformatics

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Topic: I.02. Systems Biology and Bioinformatics

Support: National Institute on Drug Abuse Intramural Research Program

Title: A bioinformatic pipeline for the discovery of translational targets relevant to cocaine abuse

Authors: *R. J. ELLIS, J. L. GOMEZ, L. A. RODRIGUEZ, M. MICHAELIDES; Biobehavioral Imaging and Mol. Neuropsychopharm. Unit, Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Science is generating data at an exponential pace, and advances in web-based computational technologies facilitate data sharing initiatives aimed at guiding scientific discovery via public sharing of such data. While such practices have been increasing in prevalence, the amount of publicly available experimental data is vast and ever-increasing, and tools that allow efficient retrieval and/or processing of accessed data is limited. Furthermore, the majority of such tools do not permit easy data accessibility and compatibility across different platforms. Here we describe the first-stage development of an automated software pipeline which integrates both human and animal phenotypic, genomic, gene expression, and disease information derived using a variety of independent web-based empirical databases. We further describe a first-stage assessment of the performance capability of our approach by examining mechanistic involvement of the predicted targets in cocaine-related behaviors. Ultimately, our goal is to develop and apply this automated pipeline to guide efforts aimed at discovering novel translational therapeutic targets relevant to substance abuse and addiction.

Disclosures: R.J. Ellis: None. **J.L. Gomez:** None. **L.A. Rodriguez:** None. **M. Michaelides:** None.

Poster

467. Bioinformatics

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Program#/Poster#: 467.07/MMM43

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant OD011190

Title: Finding neuronal cre-expressing mutant mice using www.creportal.org

Authors: *H. ONDA[1], S. A. MURRAY[2], M. KNOWLTON[1], C. L. SMITH[1], J. T. EPPIG[1]; [1]Mouse Genome Informatics, [2]Genet. Resource Sci., The Jackson Lab., Bar Harbor, ME

Abstract: Conditional mutagenesis is a powerful method for studying cell type- and stage-specific gene interactions during brain development and in neurological disease processes. Mice carrying a target gene with inserted loxP sites are mated with mice bearing cre-recombinase

transgenes or knock-in alleles. Depending upon its driver/promoter, cre recombinase is activated in a temporal and spatial-specific manner, causing excision of the genomic region flanked by the loxP sites of the target gene. This technique is particularly useful to examine gene function temporally where a conventional knock-out might result in embryonic lethality or to examine age-specific tissue expression. The CrePortal (www.creportal.org) facilitates identification of the most suitable cre mouse lines for such conditional mutagenesis experiments. It describes over 2,550 recombinase containing transgenes and knock-in alleles with detailed molecular information and tissue- and age-specific cre activity. Over 700 cre transgenes and knock-in alleles exhibit activity in the nervous system. Data on cre-expressing mice with neuronal drivers have been integrated from individual laboratories and large-scale programs including the NIH Neuroscience Blueprint Cre Driver Network, the Allen Institute for Brain Science, the Pleiades Promoter project, the JAX Cre Resources project, and EUCOMMTOOLS. Cre activity data are annotated using the Abstract Mouse (EMAPA) ontology with images of intended and off-target activity. The CrePortal can be searched by the anatomical structure in which recombinase activity was assayed and/or by the driver used to activate the cre recombinase. The search summary provides a list of drivers, the recombinase-containing allele symbols, associated gene and allele name, allele synonym, a list of tissues in which recombinase activity was detected or not detected, the inducible agent if required, links to references, and links to the International Mouse Strain Resource (IMSR, www.findmice.org) for locating those strains available through public repositories. Filters allow users to refine search results. Allele symbols are linked to the Mouse Genome Informatics Database (MGI, www.informatics.jax.org) phenotypic data pages for the cre transgene and alleles that provides a more detailed cre activity summary and phenotypic information for genotypes involving the cre transgene and allele. We encourage you to explore the CrePortal and submit your laboratory's cre line observations for inclusion at www.informatics.jax.org/submit.shtml.

Disclosures: **H. Onda:** None. **S.A. Murray:** None. **M. Knowlton:** None. **C.L. Smith:** None. **J.T. Eppig:** None.

Poster

467. Bioinformatics

Location: Halls B-H

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Program#/Poster#: 467.08/MMM44

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant 5R01AG047589-02

Title: An inter-region model of the mouse brain mesoscale connectome.

Authors: *N. S. GRADDIS[1], K. D. HARRIS[2], N. CAIN[1], J. D. WHITESELL[1], K. E. HIROKAWA[1], E. T. SHEA-BROWN[2], J. A. HARRIS[1], S. MIHALAS[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Applied Mathematics, Univ. of Washington, Seattle, WA

Abstract: Knowledge of an organism's connectome - the existence and strength of connections between individual neurons (microscale), populations of neurons (mesoscale) or larger areas (macroscale) - can guide experimentation, provide a scaffold for models of neural computation, and may help explain disease progression. The Allen Mouse Brain Connectivity Atlas is a large effort to systematically map axonal projections across the whole brain using viral tracers, high-throughput serial two-photon tomography, combined with informatics processing and registration of images to a 3-D common coordinate reference space. This pipeline results in quantitative measures of connection strengths between injection sources and target brain regions, and is currently the most comprehensive dataset for analyses on the mouse mesoscale connectome. However to characterize the connections originating from regions not injection sites, which can include neurons in multiple areas, a model is required. We previously build such a model explaining connectivity strength in each target region as a linear weighted sum of the injection volumes across injected regions, and was based on two assumptions, 1) projections can be described by the relationship between the total signal in source and target regions and 2) the contributions of multiple source regions can be linearly combined. These assumptions allow us to address the problems of incomplete injection coverage, variable injection locations, and overlap of region boundaries by injections, but was not able to provide full coverage. Here, we present an extension and update to the original linear model. Our specific goals were to expand the coverage and improve the reliability of the model. We deal with the ill-conditioning of our design matrix by employing ridge regression and account for the presence of outliers by replacing our least-squares loss term with Huber loss. When tested against presence/no presence expert classification of a subset of connections, our model achieves true positive rates of around 80% (versus 75% in the unmodified linear model) and false-positive rates around 13% (16%). These improvements to model performance allow us to more accurately, completely, and quantitatively parameterize mesoscale connectivity in an automated and high-throughput fashion. This enables, among other things, more rigorous graph theoretical analyses of large-scale network topology and more accurate simulations of population-level activity.

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Poster

467. Bioinformatics

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Program#/Poster#: 467.09/MMM45

Topic: I.02. Systems Biology and Bioinformatics

Support: BMBF Grant 01GQ1302

Title: Keeping track of your data with tools for comprehensive data organization

Authors: C. J. KELLNER[1], A. STOEWER[1], M. SONNTAG[1], A. KOUTSOU[1], A. SOBOLEV[1], J. BENDA[2], *T. WACHTLER[1], J. GREWE[2];
[1]Ludwig-Maximilians-Univ Munich, 82152 Planegg-Martinsried, Germany; [2]Eberhard Karls Univ., Tuebingen, Germany

Abstract: Making sense of complex neuroscience data requires integration of information from multiple sources. Recorded data need to be joined with metadata describing experimental conditions and analyses. Data identification and retrieval is considerably enhanced by tightly linking data with their corresponding metadata. We previously developed a format for metadata, the odML[1], and here present a data format and tools to link data and metadata meaningfully for easy data exploration, selection, retrieval, and sharing. The NIX[2] format can store various kinds of scientific data, like electrophysiological, imaging, or other recorded or derived data, together with the metadata and including relationships between data items. Data are stored with units and dimension information, for direct interpretation. The format allows specifying regions of interest, such as areas in an image or events in a recorded signal, and references between data items. Integration into the recording or analysis software is made possible through libraries for different languages, including C++, Python, Matlab, and Java. Installable packages exist for the major platforms, together with documentation, examples, and tutorials. The NixView program[3] can be used to explore, plot and export the stored data in a user friendly and convenient way. An I/O backend for the Neo Python objects for electrophysiology [4,5] enables conversion of data from various proprietary formats to the open NIX format. Moreover, results of data analysis done with Neo, for example using the Elephant[6] toolkit, can be saved in the very same format. The NIX project thus facilitates data integrity and reproducibility through comprehensive annotation and efficient organization of neuroscience data during everyday lab work.
[1] http://dx.doi.org/10.3389/fninf.2011.00016
[2] https://github.com/G-Node/nix
[3] http://bendalab.github.io/NixView/
[4] http://neuralensemble.org/neo/
[5] https://github.com/G-Node/python-neo-nixio
[6] http://neuralensemble.org/elephant/

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Poster

467. Bioinformatics

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: I.02. Systems Biology and Bioinformatics

Support: NICHD R01 HD057632

The Miami Project to Cure Paralysis

The Buoniconti Fund

The University of Miami Center for Computational Science Fellows Program

The Walter G. Ross Foundation

Title: Inferring "driver" transcription factors from RNA-Seq data

Authors: ***M. DANZI**[1], J. L. BIXBY[2], V. P. LEMMON[2], S. WUCHTY[3];
[2]Miami Project to Cure Paralysis, [3]Dept. of Computer Sci., [1]Univ. of Miami, Miami, FL

Abstract: Data science approaches to analyzing gene expression datasets often involve derivation of a "signature", comprising a set of genes consistently regulated in the experimental or diseased condition. These analyses generally find patterns that fit the data at hand, but do not generalize to new datasets. This non-generalizability may arise because gene expression profiling is measuring the "passengers" rather than the "drivers" of the biological event being studied. We hypothesize that identification of "driver" genes will lead to greater consistency and thus generalizability in the patterns discovered in biological datasets. One major, well-studied class of "drivers" affecting gene expression comprises transcription factors. Therefore, investigators have proposed methods to use information about the activities of transcription factors in conjunction with RNA-Seq data to derive the "driver" transcription factors responsible for observed changes in gene expression. Here, we propose a new algorithm for this purpose. The algorithm uses a decision tree structure to group genes into similarly regulated cohorts based on the degree to which their expression changes across experimental conditions and the combinations of transcription factors proposed to regulate them. This approach can also be combined with epigenetic data describing the location of regions of accessible chromatin in the cell type of interest. We find that filtering the underlying transcriptional regulatory network to include only

interactions in which the target DNA is accessible further enhances the performance of this algorithm.

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Poster

467. Bioinformatics

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Topic: I.02. Systems Biology and Bioinformatics

Support: GEBIOMIC Group

Title: Structural analysis of membrane myelin zero and mannose binding proteins from neurons, that modulate immune response against Mycobacterium leprae infection

Authors: ***M. CORREDOR**[1], V. CARDONA[3], A. MUÑOZ-GOMEZ[2];
[2]GEBIOMIC group, [1]Univ. of Antioquia, Medellín, Colombia; [3]Univ. of Antioquia, Biol. Inst., Medellín, Colombia

Abstract: Introduction: Leprosy is a chronic infectious disease that invade neurons caused by Mycobacterium leprae infection to severe neuronal damage. M. leprae has the capacity to invade the peripheral nervous system and cause neuropathy. The molecular mechanisms responsible have begun to be clarified: Myelin protein Zero (MPZ) is a major structural component of the myelin sheath, and its deficiency due to various mutations in the MPZ gene; Mannan-binding lectin (MBL) activates the complement system via the lectin pathway and was associate with Alzheimer and Schizophrenia diseases . Genetic and environmental factors favor infection and disease development. Mutations in allele's MPZ and MBL proteins and lead to nonsynonymous changes that alter some points binding proteins and their structures; as myelin protein zero and C mannose binding protein, respectively. Structural analysis allows to study the putative amino acids regarding their possible effect on structure and function, as well as sequences which are part of these gene variants and their value within the protein. **Methods:** nucleotide sweep was initially performed in patients from three Colombian populations with the disease SNPs obtained in exons of two genes, MPZ and MBL. Additionally a measure of synonymous mutations was performed and nonsynonymous using the KaKs tool that evaluates significant differences in nonsynonymous and synonymous mutations. Subsequently, an analysis of the domains with the Muscle, Hammer, tools Hmmerview compared with SMART databases. Then the protein structures built using the server I-TASSER and structural alignments with the tool are effected Chimera. **Results:** At nonsynonymous mutations observed in both proteins apparently not affect

the structure but such amino acid changes affect the net charge of some regions conferring differential to the putative functions. By making a structural analysis of these two proteins it is determined that such mutations fall on seemingly minor structural zones (loops). These loops or loop regions play an important role. In the case of MPZ mutation in amino acid on the KNPPD loop (T / I) VGK threonine (T) by isoleucine (I) does not change the loop charge but makes this region exposed to the outside of the membrane more hydrophobic by isoleucine. For the case of MBL amino acids change in a rich giant loop glycine reveals much more drastic changes especially in charge on the GTK (G / E) EKGE sequence, carrying the loop from neutral to acidic (- 1), that happens because glycine (G) to glutamic acid (E) mutation.

Disclosures: **M. Corredor:** A. Employment/Salary (full or part-time): University of Antioquia, Biology Institute, GEBIOMIC Group. **V. Cardona:** None. **A. Muñoz-Gomez:** None.

Poster

467. Bioinformatics

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NSF DBI-1546

NSF DBI-11471

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A*STAR JCO1231BFG040

Title: BigNeuron algorithm porting and bench testing for automatic, massive-scale neuron reconstruction

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Abstract: Neuron reconstruction from microscopic images is recognized as one of the major technical challenges in the digital era of neuroscience. The BigNeuron project (Peng, Hawrylycz et al. Neuron, 2015, DOI: 10.1016/j.neuron.2015.06.036; Peng, Meijering et al. NeuroInformatics, 2015, DOI: 10.1007/s12021-015-9270-9) is a global effort to standardize the interfaces of algorithms and neuron image data, and to provide a common platform to facilitate fair, high-throughput bench testing of various neuron reconstruction methods. We have chosen to use Vaa3D (Peng, Ruan et al. Nature Biotechnology, 2010, DOI:10.1038/nbt.1612, 2010; Peng, Bria et al. 2014, Nature Protocols, DOI: 10.1038/nprot.2014.011) as the software platform for researchers and developers to port their methods, followed by comparing and analyzing such reconstruction algorithms. Specifically, we have leveraged Vaa3D's unique open source plugin interface to help developers quickly port their algorithms in the course of a number of hackathons in China, UK, and USA during 2015~2016.
With the community contribution from about 200 researchers, more than 30 reconstruction algorithms have been ported onto Vaa3D as standalone plugins with a standardized software and data interface. These algorithms cover several major known categories of methods, including structure pruning, fitting geometrical elements, ray casting, spanning trees and shortest paths, integer programming, linear programming, deformable curves, machine learning, 2D to 3D mapping, tips finding, and others.
With about 10 million CPU hours provided by the supercomputing facilities at the Oak Ridge National Lab, the Lawrence Berkeley National Lab, and the Allen Institute for Brain Science, we bench tested all ported algorithms versus all contributed neuron image data. So far, for almost 40,000 single neuron image stacks including human, mouse, rat, fly, chicken, frog, zebrafish, etc., we have generated over 2.6 million neuron reconstructions including about 1.5 million reconstructions for explorative testing and another 1.1 million reconstructions with refined

implementation of the ported algorithms. All these reconstructions will be further analyzed and the results will be shared with the entire neuroscience community as a useful new resource.
Support: The Office of Science of the U.S. Department of Energy No. DE-AC05-00OR22725 (Oak Ridge National Lab), NIH R01NS39600 (GAA), NIH R01NS086082 (GAA), NSF IIS-1302256 (GAA), NSF DBI-15463 (GAA), NSF DBI-1147134, NSF DBI-1350258, A*STAR JCO1231BFG040 (LC, GL),MOST 104-2221-E-011 -085 (CWW),Chinese Natural Science Foundation Project #61201396 #U1201255(LQ).

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Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 467.13/MMM48

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH R01NS39600 (GAA)

NIH R01NS086082 (GAA)

NSF IIS-1302256 (GAA)

NSF DBI-15463 (GAA)

Title: Bigneuron data analysis for massive-scale, automated neuron reconstructions

Authors: ***X. LIU**[1], Z. ZHOU[1], T. GILLETTE[2], G. ASCOLI[2], M. HAWRYLYCZ[1], S. HILL[3], C. KOCH[1], E. MEIJERING[4], H. PENG[1];
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polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland; [4]Erasmus Univ. Med. Ctr., Rottertham, Netherlands

Abstract: BigNeuron [1] is a worldwide community project to advance the research and applications of single neuron reconstruction, which remains to be an important challenge in brain science. So far, the BigNeuron project has bench-tested 38,769 contributed neuronal microscopy image stacks from worldwide institutes, and in the current second version of batch testing the project has generated 1,110,590 reconstructions from more than 30 contributed neuron tracing algorithms.

To evaluate the automatic tracing results quantitatively, we have calculated morphological properties/features (such as BlastNeuron [2] metrics) and spatial distances to measure the accuracy of the reconstructions with respect to manually curated reconstructions (the gold standard) and to compare the differences between the reconstructions from the same image datasets. Due to the diversity of the BigNeuron image datasets and the different engineering designs of BigNeuron algorithms, the performance ranking of the algorithms is quite different across different image datasets. For images that are relatively challenging due to lower signal-to-noise ratio or intense imaging artifacts, the reconstructions from different algorithms tend to capture different characteristics of the underlying neuron morphology.

In order to leverage all the automatic tracing results and produce faithful representative reconstructions for all BigNeuron images, we have developed an algorithm to fuse multiple reconstructions into a single consensus reconstruction for each image based on the neuronal skeleton locations and connections agreed upon by the majority of the input reconstructions. When comparing the consensus results to individual auto reconstructions on a dataset of 166 images with gold standard reconstructions, we found that the consensus is closer to the gold standards than each and any of the individual algorithms. For another dataset of more than 25,000 images, of which no gold standards are provided, we found that the consensus is closer to the population center (has a smaller total distance to individual reconstructions) than the median case reconstruction for each dataset.

[1] "BigNeuron: large-scale 3D neuron reconstruction from optical microscopy images" ,Peng, H., Hawrylycz, M., Roskams, J., Hill, S., Spruston, N., Meijering, E., Ascoli, G.A Neuron,Vol. 87, No. 2, pp. 252-256, 2015.[2] "Blastneuron for automated comparison, retrieval and clustering of 3d neuron morphologies", Wan, Y., Long, F., Qu, L., Xiao, H., Hawrylycz, M., Myers, E., Peng, H., NeuroInformatics, 2015,13(4):487-99

Disclosures: **X. Liu:** None. **Z. Zhou:** None. **T. Gillette:** None. **G. Ascoli:** None. **M. Hawrylycz:** None. **S. Hill:** None. **C. Koch:** None. **E. Meijering:** None. **H. Peng:** None.

Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

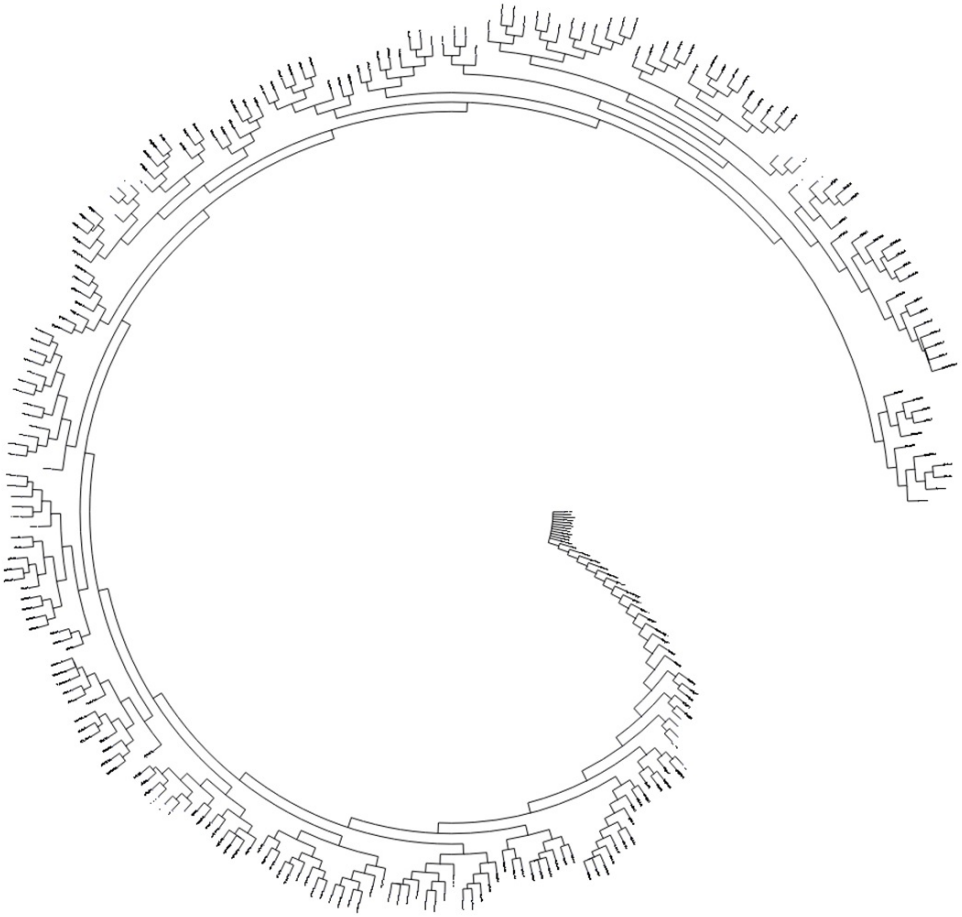
Program#/Poster#: 467.14/MMM49

Topic: I.02. Systems Biology and Bioinformatics

Title: Bioinformatic analysis of phenotypic data of ASD rodent models.

Authors: *W. PEREANU[1], M. A. ESTEVEZ[1], I. DAS[1], S. B. BASU[2];
[1]Mindspec, Mc Lean, VA; [2]Mindspec, McLean, VA

Abstract: Background: The Autism Database (AutDB) is a publicly available, manually annotated, modular database that serves as an ongoing collection of genes linked to Autism Spectrum Disorders (ASD). The animal model module of AutDB catalogues over 600 ASD-related rodent models, extracted from primary literature.
Objectives: Although there have been other comparative analyses of ASD rodent models, they have been limited in scope, both in terms of the number of animal models and the extent of phenotypic assessments used. By looking at the total data sets of rodent models that is available in AutDB, we are expanding our analysis to more than 600 ASD rodent models, and about 375 phenotypic parameters, divided in 16 larger categories. A bioinformatics analysis of this scope can be used to elucidate ASD research trends and etiology
Methods: All data is extracted from published, peerreviewed primary reports. The metadata is standardized in a phenotypic database, which is a routinely updated comprehensive list of phenotypic terms (phenoterms) and experimental paradigms. These phenoterms reflect the actual research and are divided into categories that align with human ASD phenotypic features. For each individual model, annotated phenoterms contain a given value (e.g. increased, decreased, no change, abnormal). Using the aggregate of these phenoterm values, models are clustered into functional groups.
Results: ASD rodent models cluster based on phenotypic data that reflect neurophysiological, behavioral and developmental complexity. By looking at a broad genetic and environmental model set we are able to ascertain common underlying biological mechanisms in ASD etiology.
Conclusions: The AutDB animal model module serves as a detailed repository of rodent model phenotypes reported in the ASD field. The scientific standardization of phenotypic parameters allows for data mining and bioinformatics analysis. Our present analysis provides a glimpse of the complexity of ASD etiology, and allows us to visualize the contribution of both genetic and environmental factors by using animal models.



Disclosures: **W. Pereanu:** None. **M.A. Estevez:** None. **I. Das:** None. **S.B. Basu:** None.

Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 467.15/MMM50

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant R01 GM076990

Title: Comparison of single cell and pooled cell expression data from mouse and human brain

Authors: ***O. MANCARCI**, L. TOKER, P. PAVLIDIS;
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Transcriptomics-based approaches are becoming increasingly popular for characterizing different cell types. Due to limited sensitivity of classical gene expression studies, transcriptomics-based classification was originally applied to pooled cell samples, using canonical markers for their isolation and purification. Recent developments in single cell RNA sequencing (scRNAseq) techniques allow classification of cell types based on post-hoc clustering of individual cells based on similarity in their gene expression patterns. However, despite the existence of multiple transcriptomics-based studies targeted towards classification of cell types in similar tissues (e.g. cerebral cortex), the concordance of these studies is still unclear. We sought to evaluate 1) the similarity between the clusters identified in individual scRNAseq datasets 2) correspondence between pooled-cell-based and single-cell-based cell type classification and 3) correspondence between expression profiles based on mouse vs. human cell-type specific expression data.
To address these questions we compared multiple datasets of publically available mouse and human scRNAseq data alongside data from Neuroexpresso - a rigorously curated cell-type specific expression database of pooled cell types in mouse brain compiled within our lab. Our results show that while major cellular clusters (such as oligodendrocytes, pyramidal cells) are highly similar across datasets, this similarity is decreasing with increased classification resolution. Namely, specific cellular clusters found in individual scRNAseq dataset was often absent from other scRNAseq datasets. Comparison of mouse and human data emphasizes the similarities and the difference between the two species. For example, despite the general similarity among genes highly enriched in major brain cell types, we found that about 1/3 of genes highly expressed in mouse astrocytes are not detected human astrocytes.
Evaluating the robustness of cell-specific expression data and their applicability for analyses in different species is crucial for future studies of the brain.

Disclosures: **O. Mancarci:** None. **L. Toker:** None. **P. Pavlidis:** None.

Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 467.16/MMM51

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Neuroscience Blueprint U24DA039832 via NIDA

NIH NIDDK under grant U24DK097771

Title: SciCrunch: A cooperative and collaborative data, information and resource discovery portal for scientific communities

Authors: *J. S. GRETHE[1], A. E. BANDROWSKI[1], M. CHIU[1], T. H. GILLESPIE[1], J. GO[1], Y. LI[1], I. B. OZYURT[1], L. MARENCO[2], P. L. MILLER[2], R. WANG[2], G. M. SHEPHERD[2], M. E. MARTONE[1];
[1]Ctr. for Res. in Biol. Systems, Univ. of California San Diego, LA Jolla, CA; [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract: SciCrunch was designed to allow communities of researchers to create focused portals that provide access to resources, databases, information and tools of relevance to their research areas. The SciCrunch data and information discovery index is one of the largest aggregations of scientific data, information, and tools available on the Web. Just as you can search across all the biomedical literature through PubMed, regardless of journal, SciCrunch lets you search across hundreds of databases and millions of records from a single interface. SciCrunch was designed to break down the traditional types of portal silos created by different communities, so that communities can take advantage of work done by others and share their expertise as well. When a community brings in a data source, it becomes available to other communities, thus ensuring that valuable resources are shared by other communities who might need them. At the same time, individual communities can customize the way that these resources are presented to their constituents, to ensure that their user base is served. SciCrunch currently supports a diverse collection of communities, each with their own data needs: The Neuroscience Information Framework (NIF) - is a biological search engine that allows students, educators, and researchers to navigate data resources relevant to neuroscience. NIF is the core community from which SciCrunch was developed; NIDDK Information Network (dkNET) - serves the needs of basic and clinical investigators by providing seamless access to large pools of data relevant to the mission of The National Institute of Diabetes, Digestive and Kidney Disease (NIDDK); Research Identification Initiative (RII) - aims to promote research resource identification, discovery, and reuse to improve and enhance reproducibility in scientific research; Drug Design Data Resource (D3R) - aims to advance computer-aided drug discovery through the interchange of protein-ligand datasets and workflows, and by holding community-wide challenges.

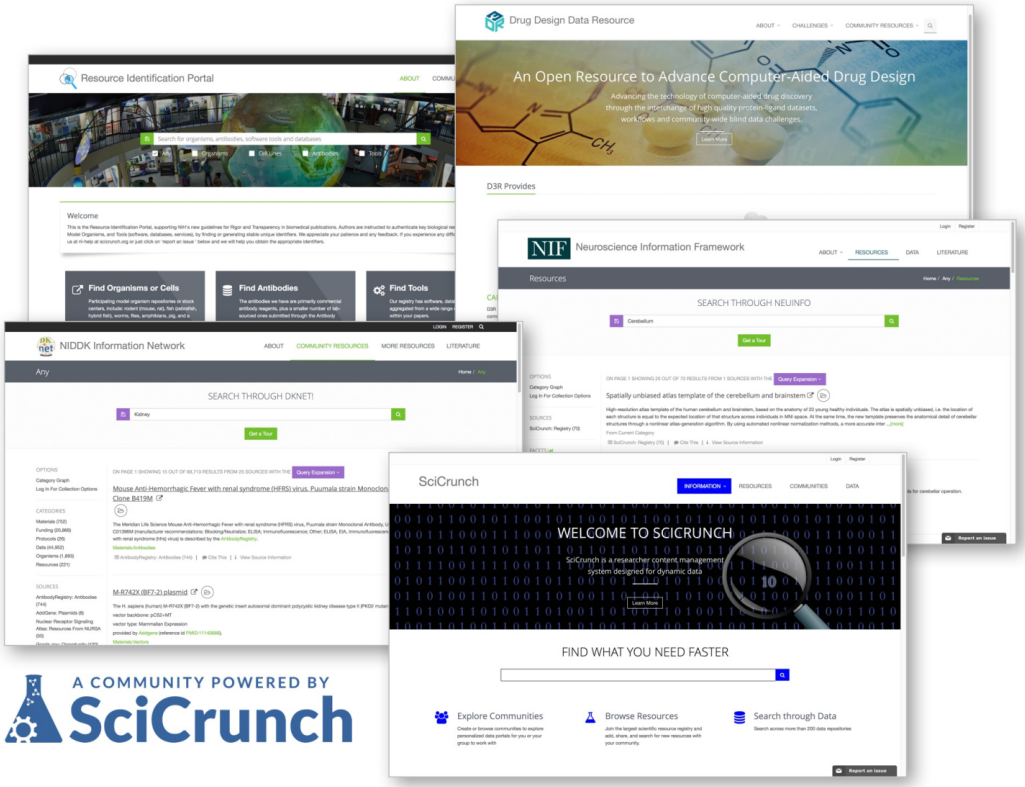


Figure 1: SciCrunch communities depicting discovery functionality

Disclosures: **J.S. Grethe:** None. **A.E. Bandrowski:** None. **M. Chiu:** None. **T.H. Gillespie:** None. **J. Go:** None. **Y. Li:** None. **I.B. Ozyurt:** None. **L. Marenco:** None. **P.L. Miller:** None. **R. Wang:** None. **G.M. Shepherd:** None. **M.E. Martone:** None.

Poster

467. Bioinformatics

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 467.17/MMM52

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant DA028420

Title: Mouse Phenome Database: A curated and integrated resource for studying sex differences and sex x genotype interactions.

Authors: *M. BOGUE, S. C. GRUBB, V. PHILIP, E. J. CHESLER;
The Jackson Lab., Bar Harbor, ME

Abstract: The Mouse Phenome Database (MPD; phenome.jax.org) is a widely used online resource providing access to primary experimental data, protocols and analysis tools for mouse phenotyping studies. Data are contributed by investigators around the world and represent a broad scope of behavioral endpoints and disease-related characteristics in naïve mice and those exposed to drugs, environmental agents or other treatments. Most data in MPD are from inbred strains and other reproducible strains such that the data are cumulative over time and across laboratories. MPD provides an important venue for compliance with data sharing policies and facilitates data reuse and data integration to provide a means of assessing replicability and reproducibility across experimental conditions and protocols. MPD has consistently provided rigorous curation of experimental data and supporting documentation. The breadth of data in MPD is amenable to the exploration of sex differences and sex x genotype interactions across many diverse phenotypes. For example it has been suggested that female laboratory animals must be monitored for estrous cycle in order to control for variation. Using a large quantity of MPD trait data from many strains of mice, we found that differences in variation are not statistically significant in randomly cycling females compared to males, suggesting that it is not necessary to monitor estrous cycle and that sample sizes of females do not need to be quadrupled in order to control for variation. Furthermore, our analysis shows there is significantly more variation in both males and females for behavioral phenotypes compared to either morphological or physiological phenotypes.

Disclosures: **M. Bogue:** None. **S.C. Grubb:** None. **V. Philip:** None. **E.J. Chesler:** None.

Poster

468. Optical Methods: Probe Development and Applications

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 468.01/MMM53

Topic: I.04. Physiological Methods

Support: NIH 1U01MH109091-01

Title: Sparse, strong and large area targeting of genetically encoded voltage indicators

Authors: S. ANTIC[1], C. SONG[2], *T. KNOPFEL[2];
[1]UConn Hlth., Farmington, CT; [2]Imperial Col. London, London, United Kingdom

Abstract: Understanding the dynamic activity of neural circuits depends on large scale recording of electrical signals produced by neurons. Large scale voltage imaging should be compatible with experiments in behaving animals and minimally invasive. Genetically encoded voltage indicators (GEVI) are arguably the most promising technique for in vivo optical monitoring of electrical activity in many neurons simultaneously. During recent years a variety of GEVIs have been developed and some of them are now expressed in transgenic mice under regulatory sequences that facilitate cell class-specific voltage imaging ex vivo and in vivo. However, current transgenic expression approaches result in a dense expression pattern. Since GEVIs fluorescence intensity depends on the area of indicator-expressing plasma membranes within the tissue voxel from which photons are sampled (and not cytosolic volume as in the case of calcium indicator), the dominant fraction of the optical signal arises from dendrites and axons. As the optical signals from many neighbouring neurons blend together in each voxel, efficient allocation of signals to individual cells is challenging. To address this issue we developed a strategy for genetic targeting sparsely but at high expression levels (so that only a single or very few cells contribute to each voxel signal), making it possible to allocate the optical signals to individual cells under conditions suitable for high frame rate wide field imaging and low optical resolution one photon and two photon laser scanning-based imaging. Importantly, our approach does not require the use of viruses but instead is based on controlling the activation of destabilized Cre (dCre) by titrated application of the antibiotic Trimethoprim (TMP). Cre-mediated recombination in a TMP dose dependent fraction of cells - within a subpopulation determined by the regulatory sequences for dCre expression - then activates GEVI expression under a strong promoter.

Disclosures: **S. Antic:** None. **C. Song:** None. **T. Knopfel:** None.

Poster

468. Optical Methods: Probe Development and Applications

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 468.02/MMM54

Topic: I.04. Physiological Methods

Support: NIH/NINDS Grant U01 NS090565-02

DARPA Grant D16PC0041

Title: Engineering and characterization of genetically encoded red and far-red voltage indicators for imaging neuronal activity

Authors: *M. KANNAN[1,2], G. VASAN[1,2], A. YANG[1], V. A. PIERIBONE[1,2];
[1]The John B Pierce Lab., New Haven, CT; [2]Cell. and Mol. Physiol., Yale Univ., New Haven, CT

Abstract: Genetically encoded calcium indicators (GECIs) are revolutionizing the way we study the brain by offering a non-invasive means to monitor neuronal activity. However, the slow kinetics of neuronal calcium responses and the inability of GECIs to report inhibitory potentials, limit their overall usefulness. Genetically encoded voltage indicators (GEVIs), on the other hand, directly capture changes in membrane potential and hence, offer a faster readout of action potentials as well as of sub-threshold excitatory and inhibitory membrane potentials. Here, we report the identification of a palette of GEVIs consisting of red and far-red fluorophores fused to different voltage-sensing domains (VSDs), including the *Ciona intestinalis* VSD and microbial opsins. These scaffolds were evolved using multiple rounds of site-directed mutagenesis combined with high-throughput screening to select for the largest size of the voltage-dependent fluorescent response (ΔF). The constructs that exhibited the largest ΔF were subsequently electroporated *in utero* into the mouse neocortex, and acute brain slices from adolescent mice were subjected to whole-cell patch clamp experiments with concomitant wide-field or two-photon imaging. The red GEVIs were compared with each other, as well as with other voltage indicators such as Arclight and Ace2N-mNeon, for brightness and membrane localization, signal size, signal-to-noise ratio, kinetics and the ability to resolve fast trains of action potentials in pyramidal neurons. Given the high tissue penetrability, with lower susceptibility to absorption or scattering as compared to GFP-based probes, red-shifted GEVIs may represent a whole new generation of voltage indicators for imaging deep brain activity.

Disclosures: M. Kannan: None. **G. Vasan:** None. **A. Yang:** None. **V.A. Pieribone:** None.

Poster

468. Optical Methods: Probe Development and Applications

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 468.03/MMM55

Topic: I.04. Physiological Methods

Support: ERC Adv MULTIGEVOS 339620

Title: Genetically encodable hybrid optical voltage sensing with high temporal resolution in neurons using a novel quencher.

Authors: *M. PABST[1], T. ALICH[1], B. SZALONTAI[1], P. TRAN[1], G. C. FAAS[2], I. MODY[1,2];
[1]Univ. of Bonn Med. Ctr., Bonn, Germany; [2]Neurol., The David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Optical voltage sensing using genetically expressed probes is highly desirable for large scale recordings of neuronal activity. The presently available genetically encodable Ca^{2+} indicators (GECI) are not well-suited for accurate detection of single action potentials (APs) and are unable to record membrane hyperpolarizations or depolarizations below AP threshold. The genetically encodable voltage indicators (GEVI) currently in use also have several drawbacks including slow response, low fluorescence, or excessive bleaching. The hybrid voltage sensing approach uses a genetically encoded fluorophore targeted to the membrane and a small quencher molecule that moves in the membrane in a voltage-dependent manner to quench or unquench the fluorophore. The most widely used quencher is dipycrilamine (DPA). However, this molecule presents several drawbacks including its explosive properties, large capacitive load on the membrane, and a narrow absorption spectrum. We have set out to search for molecules to replace DPA in the hybrid voltage sensing approach. After testing several compounds, we identified an inorganic dye (D3) with an absorption spectrum comparable to that of DPA. In hybrid voltage sensing experiments, D3 outperformed DPA in every aspect studied. Whereas DPA significantly increased membrane capacitance at a concentration of 3-5 μM, as previously reported, D3 at 20 μM did not produce significant changes in resting membrane potential, input resistance, membrane capacitance, AP threshold or width of AP at half amplitude. When optically measuring the voltage signal with 10 μM D3 in cultured mouse or rat cortical neurons expressing membrane-targeted GFP, both hyperpolarizations and depolarizations below AP threshold could be recorded with a significantly more linear voltage response than that given by DPA. Optical recordings sampled at >1,300 s^{-1} accurately reflected the shapes and amplitudes of APs elicited in the cultured neurons by depolarizing steps in whole-cell current-clamp recordings. One of the possible advantages of D3 was observed in an optical recording lasting over 60 min after the dye has been washed out from the media surrounding the cells. This indicates that D3 remains in the membrane in the vicinity of the fluorophore for a significant amount of time even after washout. This property will most likely enable its future use *in vivo*. Our findings indicate that the hybrid voltage sensor method with compounds other than DPA may hold great promise for the GEVI approach, possibly even for fluorophores spanning over a wide range of emission wavelengths.

Disclosures: **M. Pabst:** None. **T. Alich:** None. **B. Szalontai:** None. **P. Tran:** None. **G.C. Faas:** None. **I. Mody:** None.

Poster

468. Optical Methods: Probe Development and Applications

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 468.04/MMM56

Topic: I.04. Physiological Methods

Support: Marie Curie

Neurocure Center grant

DFG

Title: Characterization of the neural activity integrator CaMPARI for all-optical functional connectivity mapping in acute brain slices

Authors: *T. A. ZOLNIK[1], E. SCHREITER[2], F. JOHENNING[1], L. LOOGER[2], M. LARKUM[3], R. SACHDEV[3];
[1]Charité Universitätsmedizin, Berlin, Germany; [2]Janelia Res. Campus, Ashburn, VA; [3]Humboldt Univ., Berlin, Germany

Abstract: The calcium-modulated photoactivatable ratiometric integrator CaMPARI (Fosque et al., 2015) facilitates the study of neural circuits by permanently marking cells active during user-specified temporal windows. Permanent marker fluorescence enables tissue preservation, recording signal from large swathes of the sample, and easily correlating functional readout with other labels such as proteins and nucleic acids. One potential application of CaMPARI is labeling neurons downstream of specific populations targeted for optogenetic stimulation, giving rise to all-optical functional connectivity mapping. Here, we characterize the response of CaMPARI to several common types of neuronal calcium signals (action potentials and synaptic inputs) in mouse acute cortical brain slices. Our experiments show that CaMPARI is not only effectively converted by spikes but also by synaptic inputs. There is little photoconversion in the absence of extracellular calcium or neuronal activity. In addition, at low photoconversion light levels CaMPARI offers a wide dynamic range due to slower conversion rate; at high light levels conversion is more rapid and sensitive to activity. With these characteristics of CaMPARI defined, we stimulated posteromedial (POm) thalamic axons with channelrhodopsin-2 activation to map their functional connectivity with CaMPARI-expressing cortical neurons in acute brain slices. We found that stimulation of POm axons triggers robust photoconversion of layer 5 cortical neurons and weaker conversion of layer 2/3 neurons. Thus, CaMPARI enables network-wide, all-optical functional circuit mapping. We are using this technique to map the functional connectivity of primary motor cortex, POm, ventroposteriormedial thalamus, and layer 6B (layer 7).

Disclosures: **T.A. Zolnik:** None. **E. Schreiter:** None. **F. Johenning:** None. **L. Looger:** None. **M. Larkum:** None. **R. Sachdev:** None.

Poster

468. Optical Methods: Probe Development and Applications

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Sanda & Jeremiah Lambert ‘55 Award

Medical Research Foundation (FRM)

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Title: Development of fast-GCaMP indicators for neuronal spike counting.

Authors: *M. C. APPLEGATE[1,2], N. A. REBOLA[4,5], K. A. COUCHMAN[4,5], M. KISLIN[1,3], D. BAKSHINSKAYA[1,3], L. A. LYNCH[1,3], D. A. DIGREGORIO[4,5], S. S.-H. WANG[1,3]; [1]Princeton Neurosci. Inst., [2]Dept. of Chem., [3]Dept. of Mol. Biol., Princeton Univ., Princeton, NJ; [4]Unit of Dynamic Neuronal Imaging, Inst. Pasteur, Paris, France; [5]Unité Mixte de Recherche, Ctr. Natl. de la Recherche Scientifique 3157, Paris, France

Abstract: A long-term goal of the BRAIN Initiative is to capture the information processing done in brain circuitry by tracking neuronal activity in large numbers of individual cells in a behaving animal. The messenger ion Ca^{2+} acts as a universal signal of activity in neurons, allowing its use as a proxy for neural activity. At present, the efficiency of genetically encodable calcium indicators (GECI) to extract spike activity is limited by the slow binding kinetics and nonlinearity of the indicators. We are working to improve the kinetics of Fast-GCaMPs, a family of fast-responding GECIs, through mutagenesis of the calmodulin domain and its intramolecular binding partners. Screening of novel GECI variants was performed using stopped-flow fluorimetry on purified protein, followed by *ex vivo* and *in vivo* measurements using AAV-driven expression, including a Cre-dependent conditional expression strategy. Our mutagenesis strategies have already yielded kinetic gains. Our best-performing variants exhibit sub-micromolar affinity for calcium and show up to four-fold acceleration over GCaMP6f of rise kinetics and six-fold acceleration of decay kinetics (to a half-decay time of 8 ms at 37 °C). To detect individual action potentials as discrete events, we are monitoring Fast-GCaMPs in small

neuronal processes, where action potential-evoked calcium transients have rapid kinetics. In imaging of action potential-triggered calcium transients in parallel fiber boutons in brain slices, one of our variants, GCaMP6f-RS09, had spike-evoked signals with a half-decay time of 63 ms, opening the possibility of monitoring activity at ~20 Hz. Extrapolation from data obtained using a low-affinity small-molecule indicator suggests that detection of single APs for trains ~100 Hz should be possible with a Fast-GCaMP with sufficiently accelerated kinetics.

Disclosures: M.C. Applegate: None. **N.A. Rebola:** None. **K.A. Couchman:** None. **M. Kislin:** None. **D. Bakshinskaya:** None. **L.A. Lynch:** None. **D.A. DiGregorio:** None. **S.S. Wang:** None.

Poster

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International Headache Society

Title: Two-photon *In vivo* imaging of neuronal membrane potential with VoltageFluor

Authors: *M. VANDENBERGHE[1,7], H. UHLIROVA[1,10], M. THUNEMANN[1], K. KILIC[2], C. R. WOODFORD[3], P. TIAN[2,11], P. A. SAISAN[2], C. G. L. FERRI[2], M.-H. YANG[4], M. ABASHIN[4], Q. CHENG[2], K. L. WELDY[2], Y. FAINMAN[4], G. T. EINEVOLL[12,8], S. DJUROVIC[9,13], O. A. ANDREASSEN[7], A. M. DALE[2,1], E. W. MILLER[14], R. Y. TSIEN[5,6], A. DEVOR[2,1,15];
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Abstract: Optical imaging of voltage-sensitive dyes (VSD) offers unique opportunities for obtaining minimally invasive measurements of neuronal activity in the living brain and, in principle, is ideally suited for multiplexed measurements from large volumes of brain tissue. The available VSDs, however, do not work well under 2-photon excitation limiting their utility for *in vivo* imaging in the light scattering/absorbing brain tissue. Here, we demonstrate 2-photon excitation of VF2.1(OMe).H - a novel member of the VoltageFluor family of voltage-dependent photo-induced electron transfer (PeT) sensors [1,2].

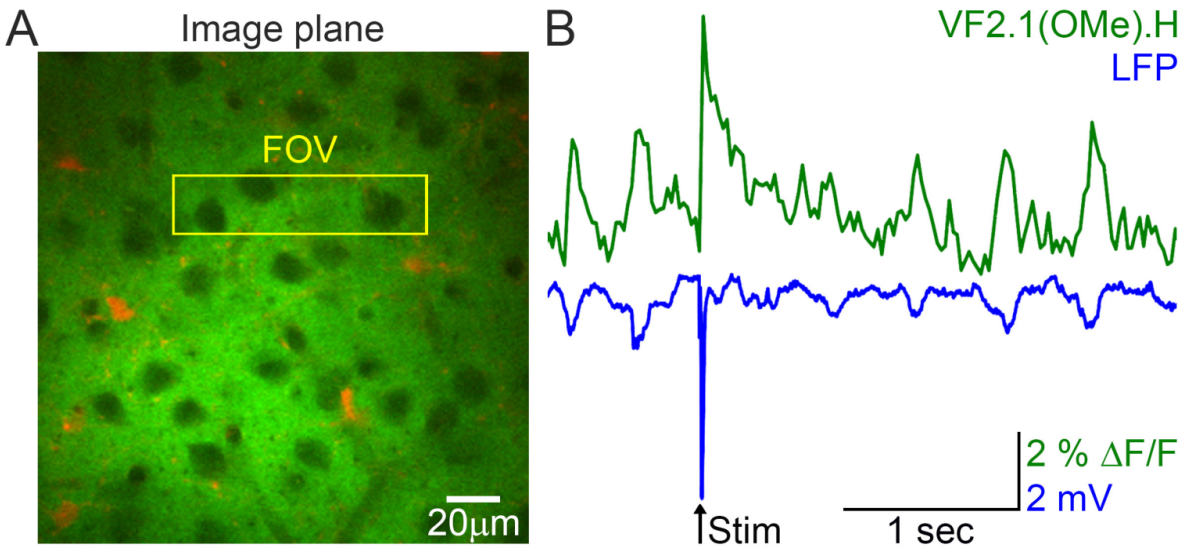
First, we combined 2-photon voltage imaging with whole-cell patch clamp recordings in cortical brain slices. The VF probe was pressure microinjected into the slice tissue through a glass micropipette. We stimulated patched neurons with 500-ms long depolarizing current pulses while imaging a ~ 50x50 μm field of view (FOV) in a frame scan mode at ~20 Hz. As expected, the spatial profile of VF signal change followed the cell boundary.

Next, we performed 2-photon voltage imaging in layer 2/3 of the mouse SI *in vivo* in response to a sensory stimulus. VF was microinjected in cortical tissue using the same procedure as in brain slices. ~ 20x100 μm FOVs were acquired in a frame scan mode at ~20 Hz. Local Field Potential (LFP) recordings were obtained simultaneously in the vicinity of the imaged FOV. Our results show that negativity in the LFP was accompanied by a simultaneous depolarization of the membranes (Figure). This is compatible with excitatory synaptic inputs and a local current sink in layer 2/3.

Thus, VF2.1(OMe).H faithfully reports neuronal membrane potential under 2-photon excitation and is well suited to obtain local voltage measurements of the neuropil *in vivo*. In the future, this type of measurements combined with depth-resolved LFP recordings would allow underpinning of the circuit behavior during evoked and spontaneous neuronal activity.

[1] Miller et al., PNAS 2012 Feb 7;109(6):2114-9

[2] Woodford et al., J Am Chem Soc. 2015 Feb 11;137(5):1817-24



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Poster

468. Optical Methods: Probe Development and Applications

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Title: Stabilizing proteins with a novel chemical technique to preserve function in unphysiological conditions

Authors: ***M. G. MCCUE**[1], Y.-G. PARK[2], H. CHOI[2], R. CHEN[2], J. YOON[2], W. TRIEU[2], K. CHUNG[2,3];
[1]Brain and Cognitive Sci. Dept., [2]MIT, Cambridge, MA; [3]Broad Inst. of Harvard Univ., Cambridge, MA

Abstract: The preservation of fluorescent protein (FP) signal is crucial in many biological studies. Chemical methods for abating loss of FP fluorescence exist but they are far from perfect. Fluorescent protein signals are highly unstable and they fade over time. FPs degrade more rapidly when they are exposed to high temperatures or harsh chemicals, conditions that are often required for many protocols in biology. Therefore, there is an urgent need for a method to increase stability of fluorescent proteins. We have developed a novel chemical treatment that incorporates our lab's recently published SWITCH concept in order to increase stability of fluorescent proteins and make them highly heat and chemical resistant. We have proven that our treatment enhances the stability of fluorescent proteins under temperatures as high as 70°C. We have also shown enhanced fluorescent protein stability under a range of harsh chemical conditions including acidic pH, detergents, and organic solvents. Finally, we have demonstrated the application of this chemical treatment as a method of preserving fluorescent protein signal during both stochastic electrotransport and passive high temperature clearing. Our method does not increase the level of autofluorescence, which has been a major issue in tissue preservation methods using glutaraldehyde. Low background autofluorescence allows for imaging of weaker signals across a wider range of fluorophore emission spectra. Ultimately, our novel chemical treatment allows for rapid tissue clearing with complete fluorescent protein preservation and significantly enhanced signal to noise ratio during imaging. This enables imaging of endogenous weak signal (such as from a viral vector injection or weakly expressing transgenic mouse line) as well as multicolor imaging to a degree which was previously not possible.

Disclosures: **M.G. McCue:** None. **Y. Park:** None. **H. Choi:** None. **R. Chen:** None. **J. Yoon:** None. **W. Trieu:** None. **K. Chung:** None.

Poster

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Title: Simple, scalable proteomic imaging for high-dimensional profiling of intact systems

Authors: *J. SWANEY[1], E. MURRAY[2], J. H. CHO[3], D. GOODWIN[6], T. KU[4], S.-Y. KIM[1], H. CHOI[4], Y.-G. PARK[4], J.-Y. PARK[4], A. HUBBERT[3], M. MCCUE[2], S. VASSALLO[5], N. BAKH[3], M. P. FROSCH[7], V. J. WEDEEDN[8], H. SEUNG[9], K. CHUNG[1];
[2]Dept. of Brain and Cognitive Sci., [3]Dept. of Chem. Engin., [4]Picower Inst. for Learning and Memory, [5]Inst. of Med. Engin. and Sci., [1]MIT, Cambridge, MA; [6]Simons Ctr. for Data Analysis, New York, NY; [7]C.S. Kubik Lab. of Neuropathology, Boston, MA; [8]Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA; [9]Princeton Univ., Princeton, NJ

Abstract: Measuring diverse molecular and structural traits over multiple length scales remains a major challenge in biology. For decades, two-dimensional molecular phenotyping techniques have been utilized for investigating tissue samples. These techniques ensure similar reaction conditions by sectioning and limiting the length scale through which reactive molecules—such as fixatives, molecular probes, and antibodies—need to diffuse. Clearing techniques such as CLARITY are able to preserve the three-dimensional spatial arrangement of endogenous molecules and enable fluorescence imaging of intact biological systems. However, slow diffusion of reactive molecules and molecular probes over system-wide length scales can cause uneven fixation and staining, respectively. Here, we introduce a simple method for the scalable, high-dimensional phenotyping of animal tissues and human clinical samples. This method, termed SWITCH, synchronizes tissue fixation across the entire system to uniformly secure the tissue architecture and native biomolecules. The preserved samples are robust to heat and chemical treatment and can be subjected to multiple rounds (>20) of relabeling. We have performed 22 rounds of labeling of a single tissue in combination with precise image co-registration. By attenuating reaction kinetics, SWITCH can also be applied to labeling reactions to improve probe penetration depth and overall staining uniformity. With SWITCH, we performed combinatorial protein expression profiling in the human cortex as well as examined the geometric structure of fiber pathways within mouse brains. SWITCH enables the extraction of high-dimensional protein expression information and may expedite our understanding of biological systems over multiple levels.

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even if those funds come to an institution; Massachusetts Alzheimer Disease Research Center. **V.J. Wedeedn:** None. **H. Seung:** None. **K. Chung:** None.

Poster

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Packard award in Science and Engineering

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NIH Grant 1-U01-NS090473-01

Title: Rapid and scalable molecular phenotyping of intact biological systems using eTANGO

Authors: *J. H. CHO[1], K. CHUNG[1,2,3,4,5];
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Abstract: Three-dimensional imaging of intact tissues has long been limited by the lack of a reliable tissue staining method. We developed a rapid and reliable labeling technique, eTANGO, that allows complete and uniform labeling of large-scale intact tissues within two days compared to weeks to months by conventional methods. eTANGO combines the SWITCH framework and stochastic electrotransport to modulate the reaction kinetics and increase the transport speed. SWITCH inhibits antibody reaction during transport into the tissue. Stochastic electrotransport rapidly transports antibodies into the tissue without damaging the tissue structure. The combined process allows all antibodies to penetrate the tissue at their maximum rates without binding to target molecules and enables complete and uniform labeling throughout the tissue. This process also works for carbohydrate binding proteins and nucleus dyes. We have successfully performed multiple rounds of labeling of the same sample using eTANGO, and have visualized 10 different cell-type markers in large-scale brain tissues. In addition, we developed an all-in-one, fully automated system to carry out the process. We envision eTANGO to accelerate discoveries in a

broad range of biological research by enabling rapid structural and molecular phenotyping of large-scale biological systems.

Disclosures: J.H. Cho: None. **K. Chung:** None.

Poster

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JPB Foundation PNDRF

NIT Grant 1-U01-NS090473-01

Title: Integrated imaging of the magnified three-dimensional proteome library of intact systems

Authors: *T. KU[1,2], J. SWANEY[3], J.-Y. PARK[1,2,7], A. ALBANESE[1], E. MURRAY[1,4], J. H. CHO[3], Y.-G. PARK[1,2], V. MANGENA[5], J. CHEN[6], K. CHUNG[1,2,3,4,8];
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Abstract: Biological processes require the coordination of multiscale interactions: from the nanoscale architecture of molecular complexes to the tissue-wide interconnectivity of cell populations. Here, we introduce a simple method for characterizing the multiscale organization of intact tissues. This method, termed MAP (Magnified Analysis of Proteome), magnifies organ architecture four-fold linearly while preserving its three-dimensional proteome organization. We discovered that preventing crosslinking within endogenous proteins during hydrogel-tissue hybridization allows for natural expansion upon protein denaturation. The magnified proteome library preserves both fine subcellular details and organ-scale intercellular connectivity. Off-the-shelf antibodies achieve multiplexed labeling and imaging of a tissue's magnified proteome with

an 82% success rate (100/122). With MAP, sample size can be reversibly modulated to accommodate proteomic imaging of inter-regional connections as well as fine synaptic architectures in the brain. The integrated multiscale mapping of the proteome within an intact tissue may enable new approaches for studying the organization and function of complex biological systems.

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Poster

468. Optical Methods: Probe Development and Applications

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Packard Award in Science and Engineering

JPB Foundation (PIIF and PNDRF)

NIH (1-U01-NS090473-01)

Title: Cell-MAP for super-resolution proteomic imaging of cultured cells

Authors: ***A. ALBANESE**[1], J. SWANEY[2], T. KU[1,3], J.-Y. PARK[1,4], K. CHUNG[1,2,3,4,5]; [1]IMES, [2]Chem. Engin., [3]Dept. of Brain and Cognitive Sci., [4]Picower Inst. for Learning and Memory, [5]Broad Inst., MIT, Cambridge, MA

Abstract: Cell-cell interactions, such as synapses, involve the coordination of organelles, proteins, and biomolecules at the nanoscale. Confocal fluorescence microscopy enables multiplexed detection of biomolecules but cannot resolve structures below the Abbe diffraction limit (~200 nm for a 63X/1.3 objective). Here, we introduce a simple method, called Magnified Analysis of Proteome (MAP), for the detailed characterization of the subcellular proteomic landscape of cultured cells. MAP processing utilizes controlled sample fixation and gel

embedding to favor protein-hydrogel over inter-protein crosslinking. Cell monolayers are captured in a polymer matrix, denatured to dissociate multi-protein complexes, and isotropically swollen in aqueous buffer. Cell-MAP preserves the three dimensional configuration of the proteome while achieving a 4-fold magnification of cell architecture. Comparison of cells before and after MAP processing with off-the-shelf antibodies reveals a remarkable improvement in the visualization of microtubules, vesicles, and organelles. Cell-MAP achieves the resolution of microtubules < 100 nm in diameter and allows us to visualize proteome architecture beneath the Abbe diffraction limit. Polymer swelling produced relatively little distortion in our embedded samples. The estimated distortion error (root-mean-square error, RMSE) was less than 3% of measured lengths at both the subcellular scale and the multicellular scale. Analysis of diversely populated cell regions confirms uniform gel expansion independent of cell density. Since our technique does not require protease digestion and preserves the spatial arrangement of proteins, we performed multi-round antibody labelling and produced super-resolution images offering unprecedented visualization of the intracellular proteome. Cell-MAP enables nanoscale characterization of organelles, vesicles, and protein complexes using conventional microscopes, common polymers, and off-the-shelf antibodies. Imaging the proteomic landscape beyond optical diffraction limits will facilitate future studies in cell and molecular biology.

Disclosures: **A. Albanese:** None. **J. Swaney:** None. **T. Ku:** None. **J. Park:** None. **K. Chung:** None.

Poster

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Program#/Poster#: 468.12/NNN2

Topic: I.04. Physiological Methods

Support: NIH DP2OD017412-01

NSF EAGER

Rita Allen Foundation

Title: A photoconvertible genetically encoded glutamate indicator for neuronal imaging

Authors: S. PAPADOPOULOS[1], J. DONG[1], J. LAMBERT[2], K. ZITO[2], *L. TIAN[1]; [1]Biochem. and Mol. Med., Univ. of California, Davis, Sacramento, CA; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Synaptic transmission is a critical event of information processing in the brain. In particular, the specific patterns of neural activity at individual synapses can drive the growth, stabilization and elimination of synaptic connections. However, how complex patterns of neural activity at multiple synapses interact to drive changes in circuit connectivity remains poorly defined. Recently, improved genetically-encoded indicators of neuronal activity have allowed for functional measurements through optical recordings of calcium, glutamate and voltage. These applications have significantly advanced the field of systems neuroscience by permitting optical recordings in specific subpopulations of neurons; however, they don't provide information to link the activity to the structural changes. Here we developed a new class of photo-convertible genetically encoded glutamate indicator that would enable one to specifically record glutamate activity in subcellular compartments, such as single spines and axonal termini within the densely labeled neurons. We demonstrate the utility of this new glutamate indicator in cells and rat hippocampal neurons. We expect that our design strategy can be applied to other types of neurotransmitter indicators in the pipeline to allow multiplex imaging of synapses.

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Poster

468. Optical Methods: Probe Development and Applications

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Topic: I.04. Physiological Methods

Support: NIH grant GG010357

NIH grant GG009410-02

Title: A novel optical probe, FFN270, enables *In vivo* multiphoton imaging of presynaptic noradrenergic synapses in mouse sensory cortex.

Authors: *S. CLARK[1], M. DUNN[2], A. HENKE[2], Y. KOVALYOVA[2], R. KARPOWICZ[2], K. KEMPADOO[3], D. SULZER[3], D. SAMES[3];
[2]Chem., [3]Neurosci., [1]Columbia Univ., New York, NY

Abstract: Norepinephrine (NE) is a monamine neurotransmitter expressed in both the central nervous system (CNS) and periphery that is important for mediating mood and maintaining homeostatic balance. Dysregulation of noradrenergic signaling has been implicated in numerous cognitive and neuropsychiatric disorders. Additionally, NE regulation is believed to control both

the depolarization and hyperpolarization of neurons in the neocortex and hippocampus, which is critical for memory and forms of synaptic plasticity. Despite these observations and the importance of NE in CNS function, there are limitations in methodology to directly explore the role of NE in normal brain function and CNS disease. Here we report the first *in vivo* optical observation of noradrenergic synapses and amphetamine's action with single synapse resolution. We utilized in-vivo multiphoton imaging of a novel norepinephrine specific fluorescent false neurotransmitter (FFN), FFN270, in mice surgically implanted with a cranial window to visualize noradrenergic release from individual synapses. We designed this FFN from a library of rationally designed, structurally related analogs and characterized it in cell culture and acute brain slice. FFN270 exhibits dual substrate activity at NET and VMAT2,. This represents the first expansion of the FFN concept beyond dopaminergic systems. For use *in vivo*, we either infused or microinjected FFN270 into barrel cortex of wild-type B6 mice, an area critical for processing sensory perception. We hypothesized that amphetamine would release NE and that the magnitude of its effect may differ between synapses. We found that in anesthetized controls, in the absence of sensory stimuli, only a small subset of noradrenergic synapses actively released FFN.. However, at 1mg/kg *d*-amphetamine (i.p.), a dosage thought to be equivalent to treatment for ADHD, the FFN was emptied from all synapses within 10 minutes. This is a first optical *in vivo* confirmation of amphetamine's role in vesicular release of NE at therapeutically relevant doses and at the level of resolution of individual synapses. Release was more rapid with 10 mg/kg a dosages consistent with amphetamine abuse. This technique far exceeds the spatial and temporal resolution of either SPECT or PET imaging, and by providing a means to study of individual synapses in vivo, elucidates the synaptic mechanisms of noradrenergic drugs.

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Poster

468. Optical Methods: Probe Development and Applications

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Heritage Medical Research Institute

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Title: Anatomical and functional characterization of wake-promoting dopamine neurons in the dorsal raphe nucleus

Authors: *J. CHO[1], J. TREWEEK[2], M. ALTERMATT[2], A. GREENBAUM[2], V. GRADINARU[2];
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Abstract: Unlike other monoamines, the role of dopamine (DA) in sleep-wake control is still ambiguous. Previous studies with pharmacological (e.g. psychomotor stimulants amphetamine and modafinil) or genetic (e.g. DA transporter knockout mice) means confer the role of DA in behavioral arousal and hyperactivity. Chemical lesion of sparse DA cells in the dorsal raphe nucleus (DRN) was observed to cause a sustained increase in sleep, while wakefulness elicited c-fos expression selectively in DA cells (Lu et al., 2006). These data, however, do not clarify whether the relationship between DRN DA activity and wakefulness is correlative, or if DA activity plays a crucial role in the entry into and maintenance of wakefulness. Here we used optical and genetic methods to investigate the role of DRN DA neurons in sleep-wake control in tyrosine hydroxylase::IRES-Cre mice. From synchronized fiber photometry (a method for monitoring bulk GCaMP6 fluorescence in deep brain regions, Lerner et al., 2015) and EEG/EMG recordings, we found that DRN DA activity increased at sleep-to-wake transitions (NREM-to-wake: 0.11 ± 0.10 versus 3.04 ± 0.39, REM-to-wake: 0.51 ± 0.2 versus 3.54 ± 0.42, in z-scored fluorescence, n=6) and at NREM-to-REM transitions (0.22 ± 0.11 versus 1.30 ± 0.16), but decreased at wake-to-NREM transitions (-0.05 ± 0.12 versus -0.73 ± 0.16). Likewise, spontaneous activity of DRN DA neurons was also highest during wakefulness. Phasic activation of ChR2-expressing DRN DA neurons induced immediate sleep-to-wake transitions (ChR2 group: +58 ± 12% versus eGFP group: -1 ± 7%, in change of wake probability, both n=6). Chemogenetic inhibition with hM4Di at the time of highest wake drive reduced the time spent in wakefulness (CNO: 47 ± 11% versus Saline: 86 ± 9%, n=4). To explore the potential ramifications of DRN DA participation in sleep-wake patterning, we performed projection mapping and found rich DRN DA innervation in the forebrain limbic structures such as medial prefrontal cortex and extended amygdala. These results open new avenues for investigation of DRN DA neurons in the allostatic or emotional regulation of sleep-wake states.

Disclosures: J. Cho: None. **J. Treweek:** None. **M. Altermatt:** None. **A. Greenbaum:** None. **V. Gradinaru:** None.

Poster

468. Optical Methods: Probe Development and Applications

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Topic: I.04. Physiological Methods

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NIH/NIA 1R01AG047664-01

Heritage Medical Foundation

Pew Charitable Trust

Title: Progression of Parkinson's-like pathology following inoculation of alpha-synuclein preformed fibrils in the gut

Authors: *C. CHALLIS[1], T. R. SAMPSON[1], B. YOO[1], S. K. MAZMANIAN[1], L. A. VOLPICELLI-DALEY[2], V. GRADINARU[1];
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Abstract: The primary hallmarks of Parkinson's disease (PD) include the formation of inclusions composed of insoluble alpha-synuclein (aSyn) fibrils known as Lewy bodies and neurites, the loss of catecholaminergic neurons, and motor impairments. However, emerging findings suggest that a prodromal phase characterized by non-motor symptoms such as gastrointestinal (GI) and olfactory deficits may precede clinical diagnosis of PD (Hawkes et al., 2010). Postmortem biopsies from asymptomatic and PD-diagnosed individuals revealed the presence of pathologic aSyn assemblies in GI and olfactory tissue, suggesting propagation of aSyn fibrils from peripheral organs to the central nervous system where they precipitate PD. This is supported by findings that aSyn fibrils are interneuronally transported and can seed the formation of additional fibrils from endogenous aSyn monomers (Volpicelli-Daley et al., 2011). Here, we inoculated stomach and duodenum lining of adult C57Bl/6 mice with aSyn preformed fibrils (PFF) and assessed GI health, motor function, and histology at 7, 21, 60, and 90 days post-PFF injection (dPPI) compared to saline-injected controls. At 7 and 21 dPPI, fecal composition and output was significantly altered, with measures returning to baseline levels by 60 dPPI. Using a battery of tests of motor strength and coordination we found significant deficits in the inverted wire hang and adhesive removal tasks at 60 and 90 dPPI. We performed PACT (PAssive CLARITY Technique) on whole, intact stomach and duodenum as well as 500µm thick brain sections and stained for phosphorylated (P)-aSyn, a pathological modification of aSyn fibrils not present on PFF. In the gut, fibril formation increased at 7 dPPI and peaked at 21 dPPI

in enteric neurons of the villi, crypts, and submucosal and myenteric plexuses, with signal diminishing to baseline levels at 60 dPPI. At 21 dPPI we observed an increase in P-aSyn signal in vagal nuclei of the brainstem and at 60 dPPI, P-aSyn was observed in the midbrain. Our results indicate time-dependent aSyn fibril propagation from the gut to the central nervous system via the vagus nerve following gut PFF inoculation that triggers early GI dysfunction and subsequent motor deficits.

Disclosures: C. Challis: None. **T.R. Sampson:** None. **B. Yoo:** None. **S.K. Mazmanian:** None. **L.A. Volpicelli-Daley:** None. **V. Gradinaru:** None.

Poster

468. Optical Methods: Probe Development and Applications

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Beckman Institute for CLARITY, Optogenetics, and Vector Engineering

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Curci Foundation

Title: Engineering novel adeno-associated viruses for enhanced transduction and target specificity across the CNS by adopting high-throughput *In vivo* and in silico methods.

Authors: *T. DOBREVA, D. BROWN, S. KUMAR, Y. LUO, R. HURT, B. E. DEVERMAN, V. GRADINARU;
Caltech, Pasadena, CA

Abstract: In recent years, adeno-associated viruses (AAVs) have found increasing success as gene delivery vehicles for basic neuroscience applications and are being evaluated in gene therapy clinical trials owing to their non-pathogenicity and transduction capabilities. To broaden their scientific and therapeutic applications, AAVs can be engineered for improved transduction

efficiency and cell-type/tissue specificity. We recently developed a Cre recombination-based AAV targeted evolution (CREATE) method to identify AAV capsid variants from capsid libraries that exhibit improved central nervous system (CNS) transduction characteristics following intravascular administration in adult mice (Deverman et al, 2015). When delivered systemically, one of the recovered variants, AAV-PHP.B, was 40-92 fold more efficient at gene transfer to the CNS compared to AAV9. One limitation of this method is that it is not feasible to recover all the enriched variants from the mouse brain using the traditional clonal sequencing. Here we report an improved methodology where we combined our selection platform with next-generation sequencing (NGS) of recovered variants as well as the starting library. This high-fidelity quantification of recovered sequences gives a quantitative understanding of the relative differences in transduction between variants. By using NGS to recover sequences from multiple Cre-expressing target cells, we are able to apply both positive selection across multiple cell types as well as negative selection to identify variants with more selective tropisms. In addition, we are using machine learning methods such as softmax logistic regression to extract the specific amino acid and nucleotide sequences from the capsid library that contributed, with highest probability, to enrichment in a specific cell type. This serves as a feedback loop, allowing us to systematically identify new variant candidates. Using a library of amino acid characteristics such as hydrophobicity, charge, and flexibility, we are testing the predictive value of our *in silico* selection pipeline by experimentally verifying the transduction of variants that do not explicitly show up in the deep sequencing results. In our pilot experiment, we achieved 80-90% accuracy in predicting whether or not a variant was highly enriched for a specific Cre-line. As a proof of principle, we are presenting some novel AAV variants that were identified using the methods described. These insights from our *in silico* methods can help elucidate what features of the viral capsid contribute to tropism, and thus help develop the next generation of gene delivery vehicles for biomedical applications.

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Poster

468. Optical Methods: Probe Development and Applications

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PECASE

Heritage Medical Foundation

Beckman Institute for the Resource Center on CLARITY, Optogenetics, and Vector Engineering

Title: Long-term tracking and automated image analysis reveals that the upside-down jellyfish has a sleep-like state.

Authors: C. N. BEDBROOK[1], R. D. NATH[1], M. J. ABRAMS[1], J. S. BOIS[1], L. A. GOENTORO[1], P. W. STERNBERG[1], *V. GRADINARU[2,1];
[1]Biol. & Biol. Engin., [2]Neurosci., CALTECH, Pasadena, CA

Abstract: Long-term behavioral tracking is an invaluable tool for investigating the full diversity of behaviors an animal can perform, especially when applied to understudied, non-traditional model organisms. We looked within the phylum Cnidaria, which diverged from Metazoa prior to the Cambrian explosion. Any shared characteristics between Cnidarians and humans represent deep evolutionary homology as their last common ancestor lived over 600 million yeas ago. Cnidaria are one of the earliest animal phyla to have evolved neurons, which organize into diffuse nerve nets. Cnidarian jellyfish *Cassiopea,* commonly known as the upside-down jellyfish, live in shallow waters and pulse for critical functions, e.g., gathering food, eliminating waste, and dispersing gametes. We built a long-term recording and tracking system to quantify the pulsation rate of *Cassiopea*. In this system we record jellyfish pulsing behavior at 15 fps followed by automated pulse counting based on changes in pixel intensity of the jellyfish in the relaxed vs contracted state. We tracked behavior over several days, with a 12/12 hr light-dark cycle. Remarkably, we found that *Cassiopea* display three hallmark behaviors of sleep: a reversible quiescent state, rebound after sleep deprivation, and reduced responsiveness to stimuli during the quiescent state. Beyond behavior, we found evidence for molecular conservation in sleep regulation. Melatonin is a known regulator of sleep across animals. We found that jellyfish pulsed less in the presence of melatonin, and resumed normal pulsation when melatonin is removed from the seawater**.** Sleep is common among vertebrates, and has been found in insects and worms. ***The discovery of sleep in a Cnidarian shifts the hypothesized root of sleep earlier in the phylogenetic tree, and raises the possibility that sleep is ancestral in the animal lineage.*** Development and application of tools for interrogating *Cassiopea's* primitive nervous system (i.e. whole animal imaging and staining) could shed light on the evolution of sleep.

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Poster

468. Optical Methods: Probe Development and Applications

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Gordon and Betty Moore Foundation Grant GBMF2809 (V.G.)

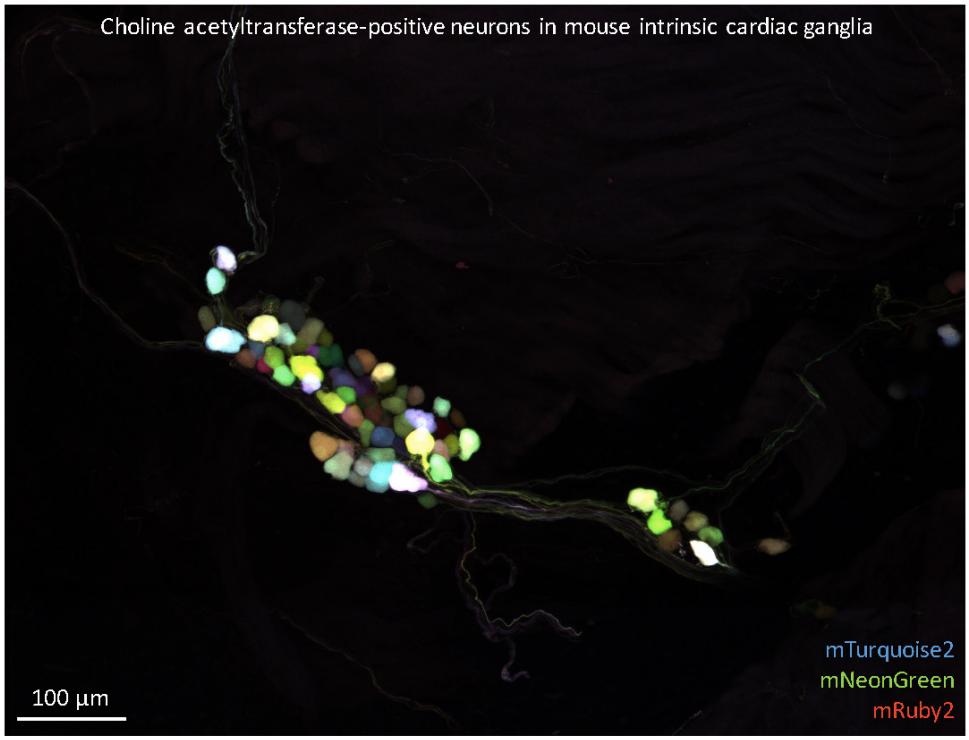
NIH/NHLBI Grant R01HL084261-02 (K.S.)

Title: Optical and viral vector strategies for mapping the structure and function of the cardiac autonomic nervous system

Authors: *P. S. RAJENDRAN[1], R. C. CHALLIS[2], K. Y. CHAN[2], B. E. DEVERMAN[2], A. GREENBAUM[2], K. SHIVKUMAR[1], V. GRADINARU[2];
[1]UCLA Cardiac Arrhythmia Ctr., Univ. of California - Los Angeles, Los Angeles, CA; [2]Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: The autonomic nervous system regulates all aspects of cardiac function and undergoes adverse remodeling in cardiac diseases. Neurons involved in mediating cardiac control are located from the insular cortex to the heart. At the organ level, the intrinsic cardiac nervous system (ICNS), a distributed network of ganglia on the surface of the heart, serves as the final common pathway for integration of neural inputs. However, the anatomical structure and function of the ICNS is not well understood, primarily due to a lack of tools that target the peripheral nervous system. Therefore, we have developed an adeno-associated virus (AAV)-based toolbox to express fluorescent proteins for multicolor labelling and anatomical characterization of peripheral neurons. This toolbox contains a novel AAV variant, PHP.S, engineered though a Cre recombination-based AAV targeted evolution (CREATE) method (Deverman et al., 2015). We utilized PHP.S and Cre-based transgenics to map the structure of the ICNS circuitry in defined cell types, including choline acetyltransferase- (Fig.), tyrosine hydroxylase- and transient receptor potential vanilloid 1-positive neurons and fibers, in the heart. To facilitate mapping of intact circuits, we employed tissue clearing techniques including PACT (PAssive CLARITY Technique) and Sca*l*eSQ together with confocal and light-sheet microscopy

to visualize transduced neurons in mouse hearts. Through this approach, we have characterized the morphology and innervation of various neuronal cell types that comprise the ICNS (e.g., sensory afferents and sympathetic and parasympathetic motor efferents). To complement our structural studies, ongoing experiments are using PHP.S to deliver opsins to intrinsic cardiac neurons to probe their function *in vivo*. This work can provide critical insights into the structure and function of the ICNS as well as the development and progression of cardiac diseases. Moreover, our novel AAV-based approach can be readily used to interrogate the morphology and function of any peripheral neural circuit that regulates organ function.



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Poster

468. Optical Methods: Probe Development and Applications

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Heritage Medical Foundation

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Title: Tissue clearing of bones for enhanced optical and molecular access to osseous neuro-immune environments using PACT-deCAL

Authors: *A. GREENBAUM[1], K. CHAN[1], T. DOBREVA[1], D. BROWN[1], D. H. BALANI[3], C. CHALLIS[1], A. LIGNELL[2], L. CAI[2], H. M. KRONENBERG[3], V. GRADINARU[1];
[1]Biol. and Biol. Engin., [2]Chem. and Chem. Engin., Caltech, Pasadena, CA; [3]Endocrine Unit, Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Many neuro-relevant circuits and pathways are embedded within difficult to access osseous tissue, which poses a fundamental limitation when conducting structural investigation using light-microscopy. The practice of bone removal (e.g. vertebrae and skull) when studying the central nervous system often leads to structural artifacts or to damage of functional structures that reside at the interface between osseous and nerve tissue. For instance, functional lymphatic vessels have been recently discovered lining the dural sinuses of the skull (Louveau et al., 2015, Aspelund et al., 2015). The unique location of these lymphatic vessels (interfaced against the typically removed skull) masked their existence, and by such hindered their discovery. This important discovery might shed light on neurodegenerative diseases linked to dysfunctions within the immune system, and highlights the significance of non-invasive structural investigation (Louveau et al., 2015, Aspelund et al., 2015).
Toward this end, we expanded upon the PAssive CLARITY Technique (PACT) (Treweek et al., 2015) to clear intact bones e.g. skull, vertebral column, femur and tibia. A key step in our proposed method is decalcification while securing tissue integrity with hydrogel embedding. The PACT-deCal technique (PACT with decalcification) retains the structure of the marrow, preserves endogenous fluorescence, and shows compatibility with small molecule dyes. Using PACT-deCal we cleared, in ~ 3 weeks, an intact mouse skull and underlying brain tissue - which allowed us to image through the skull and to observe motor and sensory neurons within the cortex (~ 500 μm deep, Thy1-YFP). Furthermore, using a custom built light-sheet microscope (LSM) we obtained multicolor images of an intact tibia and femur with micrometer resolution, and quantified the distribution of fluorescently labeled multipotent progenitor cells (endogenous fluorescence) along the bone using a high-throughput computational pipeline. The pipeline is designed for visualization, stitching and auto-detection of cell candidates in large datasets (50-500 GB). Therefore, PACT-deCal with LSM imaging provides a unique platform to study the interplay between a complex osseous tissue and neuronal circuits, as well as bone-specific phenomena such as remodeling and haematopoiesis.

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Poster

468. Optical Methods: Probe Development and Applications

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Topic: I.04. Physiological Methods

Support: Atlantic Canada Opportunities Agency

Canadian Institute for Health Research

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Title: Retinal characterization of the Thy1-GCaMP3 mouse after optic nerve transection

Authors: ***S. N. BLANDFORD**[1,2], S. R. FARRELL[1,2,4], M. L. HOOPER[1,4], B. C. CHAUHAN[1,3,4], W. H. BALDRIDGE[1,2,3];
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Abstract: The Thy1-GCaMP3 transgenic mouse has been used extensively to monitor intracellular calcium levels ($[Ca^{2+}]_i$) in the central and peripheral nervous systems, but few studies have examined the retinas of these animals. We therefore assessed which neurons in the ganglion cell layer express GCaMP3 and determined if GCaMP3 expression and response (elevated $[Ca^{2+}]_I$ produced by kainic acid) are maintained in a model of retinal ganglion cell (RGC) damage, optic nerve transection (ONT). Retinas from Thy1-GCaMP3 mice (B6; CBA-Tg(Thy1-GCaMP3)6Gfng/J; Jackson Laboratories) were examined with conventional calcium imaging. In a subset of animals, ONT was performed in one eye 3, 5 or 7 days prior to sacrifice and calcium imaging. Retinas were mounted and transient increases of $[Ca^{2+}]_i$ evoked by superfusion of kainic acid (KA; 10 μM, 50 μM, 100 μM). After live imaging, retinas were fixed and processed for immunohistochemistry with antibodies against RBPMS (an RGC specific marker) and ChAT (a selective cholinergic amacrine cell marker). Data (mean ± 95% confidence interval) were analyzed by one-way ANOVA. Baseline GCaMP3 fluorescence did not change in RGCs of ONT retinas compared to controls; however, the density of GCaMP3+ cells examined by calcium imaging was reduced 5 and 7 days post-ONT compared to control experiments (Control: 2224±1022 vs. 5 days: 1383±598; 7 days: 913±283 cells/mm^2; $p<0.05$). After axonal

injury, fewer GCaMP3+ cells responded to modest concentrations of KA compared to controls (10 μM: Control: 38%; Day 3: 7%; Day 5: 3%; Day 7: 1%; 50 μM: Control: 97%; Day 3: 54%; Day 5: 47%; Day 7: 48%). However, all cells examined responded to 100 μM KA. Ca^{2+} transient amplitudes were smaller after ONT compared to controls (10 μM KA: Control: 0.15±0.02; Day 3: 0.05±0.01; Day 5: 0.03±0.01; Day 7: 0.01±0.01; $p<0.05$; 50 μM: Control: 0.10±0.02; Day 3: 0.31±0.02; Day 5: 0.23±0.02; Day 7: 0.29±0.04; $p<0.05$; 100 μM: Control: 1.29±0.04; Day 3: 0.53±0.02; Day 5: 0.50±0.05; Day 7: 0.47±0.04; $p<0.05$). Immunohistochemical analysis demonstrated that GCaMP3 was expressed in many, but not all RBPMS+ RGCs, but not in ChAT+ amacrine cells in both control and ONT retinas. These results show that GCaMP3 is expressed in some, but not all, RGCs and shows increased fluorescence in response to KA. After ONT, the number of GCaMP3-expressing cells was decreased; however, functional responses declined in advance of RGC loss. We conclude that the Thy1-GCaMP3 transgenic mouse will be useful for future retina research, in particular because it allows for functional assessment of RGCs over time in an animal model of RGC death.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant R00NS078561

Title: Recording neuronal networks with BeRST 1, a photostable far red/near-infrared voltage sensitive dye

Authors: ***A. WALKER**[1], Y.-L. HUANG[1], E. W. MILLER[2];
[1]Chem., [2]Chemistry, Mol. and Cell Biol. & Helen Wills Neurosci. Institiute, UC Berkeley, Berkeley, CA

Abstract: Voltage sensors are an important component of the cellular neuroscience toolbox for measuring neuronal activity. They combine the direct measurement of membrane potential and high temporal resolution characteristics of electrophysiology with the highly advantageous addition of spatial information and multi-cell recording showcased by calcium imaging. We have developed BeRST 1, a novel voltage sensitive dye (VSD), which shows excellent properties for measuring neuronal activity. BeRST 1 localizes to the plasma membrane where fluctuations in

membrane potential are reported via unquenching of fluorophore emission due to photoinduced electron transfer. This mechanism acts on the nanosecond scale ensuring excellent temporal read-out of membrane potential and exerting negligible effects on membrane capacitance. BeRST 1 emits in far red/near-infrared allowing combinatorial use with widely used tools such as GFP and its derivatives (GCaMPs, ASAP 1), as well as Channelrhodopsin. The high sensitivity (24% ΔF/F per 100mV), signal-to-noise and photostability of BeRST 1 combine to allow the recording of spontaneous neuronal activity over extended imaging periods. We are employing BeRST 1 and its derivatives to simultaneously record spontaneous activity from groups of neurons and with this methodology can demonstrate the characteristic firing patterns of different neuronal subtypes, and furthermore resolve the functional connectivity of these subtypes in dissociated hippocampal culture. Current efforts are focused on applying these optical voltage sensing methods to better understand the dynamics of neural connectivity, with a special interest in understanding changes in network output and integration that occur during the pathogenesis Alzheimer's disease.

Disclosures: **A. Walker:** A. Employment/Salary (full or part-time): UC Berkeley. **Y. Huang:** A. Employment/Salary (full or part-time): UC Berkeley. **E.W. Miller:** None.

Poster

468. Optical Methods: Probe Development and Applications

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Topic: I.04. Physiological Methods

Support: NIH DP1OD003560

NIH U01NS090600

Title: High-speed recording of neural activity in awake mice and flies using a fluorescent voltage indicator

Authors: ***Y. GONG**[1,2], C. HUANG, 94305[2], J. MARSHALL[2], J. LI[2], B. GREWE[2], Y. ZHANG[2], S. EISMANN[2], M. SCHNITZER[2];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Stanford Univ., Stanford, CA

Abstract: Genetically encoded fluorescent voltage indicators (GEVIs) are a promising emerging technology for optical readout of fast neuronal spike trains and dendritic voltage dynamics. Prior GEVIs had insufficient signaling speed and dynamic range to report single action potentials in live animals. We coupled fast voltage-sensing domains (<1 ms response) from a rhodopsin

protein (Ace) to bright fluorescent proteins via fluorescence resonance energy transfer (FRET). The resulting GEVIs are sufficiently bright and fast to report action potentials and membrane voltage dynamics in the brains of awake mice and fruit flies resolving fast spike trains at rates >75 s^{-1} with 0.25 ms timing precision and spike detection error rates orders of magnitude below those of prior GEVIs. In vivo imaging in drosophila revealed sensory-evoked responses, including somatic spiking, dendritic dynamics, intracellular voltage propagation, and inter-hemispheric differences in neuronal activity. Optical monitoring of the voltage dynamics of neuronal populations in behaving mice revealed synchronized electrical activity in genetically identified cell types. These results empower in vivo optical studies of neuronal electrophysiology and motivate novel experimental designs that will allow researchers to relate high-speed neuronal dynamics and information processing to animal behavior.

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Poster

468. Optical Methods: Probe Development and Applications

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Topic: I.04. Physiological Methods

Support: NIH U01MH109146

Title: Subcellular localization of algal light-sensitive anion channelrhodopsin ACR2 and cation channelrhodopsin ChR2 in mammalian neurons

Authors: E. M. RODARTE[1], F. RIVERA-MILIAN[1], *R. JANZ[2];
[1]Neurobio. and Anat., Univ. of Texas Hlth. Sci. Ctr. Houston, Houston, TX; [2]UT-Houston Med. Schl, Houston, TX

Abstract: There has been a longstanding interest in optogenetics to develop tools that inhibit neuronal activity to complement the existing optically-sensitive cation channels (e.g. ChR2). Recently, the first anion-specific light-sensitive channelrhodopsins (ACR 1 and 2) from the algae *Guillardia theta* were isolated and proven capable of suppressing electrical activity in mammalian neurons (Govorunova et al. 2015). However, the efficiency of neuronal inhibition by anion channels is strongly dependent on the subcellular distribution of these channels. Here we analyze the subcellular localization of ACR2 expressed in mammalian neurons in comparison with ChR2. EGFP-tagged ACR2 and ChR2 were expressed under the control of the glutamatergic-neuron-specific alpha-CamKII promoter in cultured mouse hippocampal neurons

by using a lentiviral system. One week post-infection, the cells were fixed and stained with specific antibodies to determine the distribution of ACR2 and ChR2 in relation to calreticulin (ER marker), giantin (golgi marker), Na^+/K^+ ATPase (plasma membrane marker), Tau (axonal marker), MAP2 (dendritic marker), SV2, synapsin (presynaptic markers), CaMKII, VGAT (GABAergic synapse marker) and VGLUT1 (glutamatergic synapse marker). The labeled neurons were counterstained with fluorescently-labeled secondary antibodies and analyzed by confocal microscopy. We observed strong presynaptic staining of ChR2 in CAMKII+ glutamatergic neurons, which colocalizes with SV2, synapsin and VGLUT1, sharing their granular pattern along Tau-stained axons. ChR2 does not colocalize with VGAT. The soma and cytoplasm display faint diffuse staining of ChR2. These findings strongly indicate that ChR2 gets preferentially trafficked to the presynaptic plasma membrane. In contrast, ACR2 is arranged in distinct linear structures following the Na^+/K^+ ATPase lining of the plasma membrane of the soma and the dendrites, but not along the axons. It is found in close apposition to presynaptic components (SV2, Synapsin, VGLUT1, and VGAT), but does not colocalize with them. This pattern indicates that ACR2 gets preferentially trafficked to the dendritic and postsynaptic plasma membrane. No aggregates in the ER (calreticulin+) or Golgi (giantin+) were visualized on either case. Our findings suggest that endogenous targeting signals in algal ACR2 and ChR2 mediate preferential trafficking to different compartments in mammalian neurons. Further optimization will involve restricting targeting of ACR2 to axo-somatic synapses, to confer light-stimulus cell-body specificity for accurate neural network dissection.

Disclosures: **E.M. Rodarte:** None. **F. Rivera-Milian:** None. **R. Janz:** None.

Poster

468. Optical Methods: Probe Development and Applications

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Program#/Poster#: 468.24/NNN14

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Support: NSF

NAFKI

Stanford

NIH Brain Initiative

HHMI

Title: Cell-type specific optical recording of membrane voltage dynamics in freely moving mice

Authors: *J. D. MARSHALL[1], J. LI[1], Y. GONG[1], F. ST PIERRE[1], M. Z. LIN[1], M. J. SCHNITZER[2];
[1]Stanford Univ., Palo Alto, CA; [2]Applied Physics, Stanford Univ., Stanford, CA

Abstract: Electrophysiological field potential dynamics are of fundamental interest in basic and clinical neuroscience, but how specific cell types shape these dynamics in the live brain is poorly understood. To empower mechanistic studies, we created an optical technique, TEMPO, that records the aggregate trans-membrane voltage dynamics of genetically-specified neurons in freely behaving mice. TEMPO provides >10-fold better detection fidelity than prior fiber-optic techniques and attains the sensitivity limits set by quantum mechanics. After validating TEMPO's capacity to track well-established cortical and hippocampal oscillations in the delta, theta, and gamma frequency bands, we compared the dynamics of D1- and D2-dopamine-receptor-expressing subtypes of striatal medium spiny neurons (MSNs), which are interspersed and electrically indistinguishable. Unexpectedly, MSN population dynamics exhibited two distinct coherent states that were commonly indiscernible in electrical recordings and involved synchronized hyperpolarizations across both MSN subtypes. Overall, TEMPO provides general means to deconstruct the neurophysiological features of normal and pathologic brain states into trans-membrane voltage activity patterns of specific cell types.

Disclosures: J.D. Marshall: None. **J. Li:** None. **Y. Gong:** None. **F. St Pierre:** None. **M.Z. Lin:** None. **M.J. Schnitzer:** None.

Poster

468. Optical Methods: Probe Development and Applications

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 468.25/NNN15

Topic: I.04. Physiological Methods

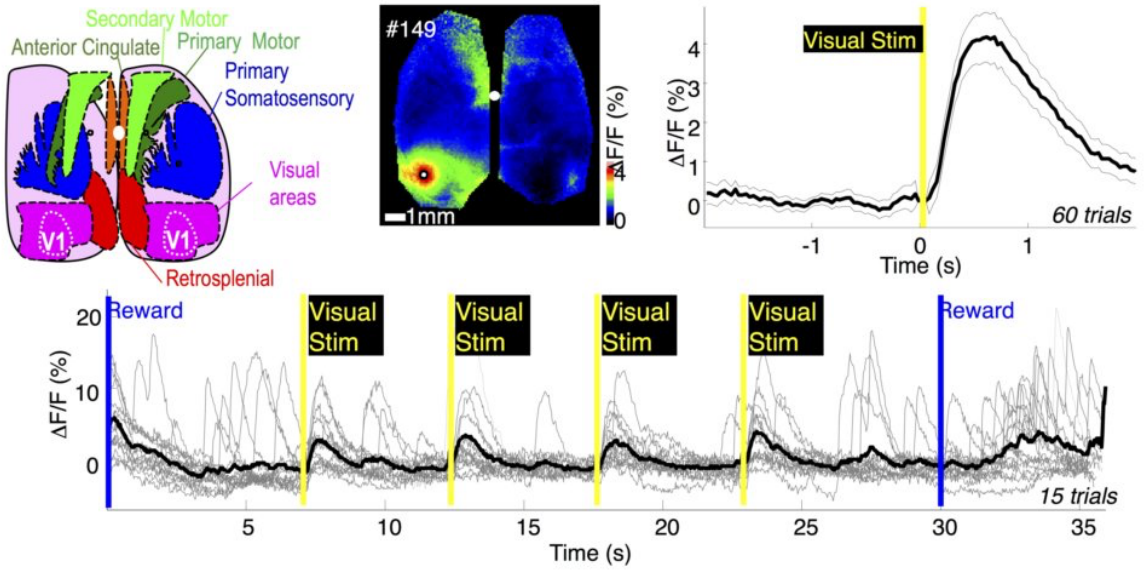
Support: CIHR Operating Grant MOP-12675

Foundation Grant FDN-143209

Title: Automated functional, mesoscopic, cortical imaging, self-initiated by gcamp6 transgenic mice in their home-cage.

Authors: *J. LEDUE, F. BOLANOS, M. VANNI, G. SILASI, J. BOYD, D. HAUPT, T. H. MURPHY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Many neuroscience experiments rely on head fixed mice to obtain optical recordings of brain activity during various behaviors or sensory stimuli of interest. Animals are often stressed upon removal from the vivarium and handling by experimenters which could introduce potential confounds. Additionally, the animals must be trained to tolerate long periods of head restraint and are usually repeatedly, individually imaged over many sessions, all of which is time intensive for the experimenter. We have developed a mouse training protocol and a home-cage based imaging system in which water restricted mice are trained to self-initiate imaging trials in exchange for water rewards. Up to five Ai93 (GCaMP6f) or Ai94 (GCamP6s) transgenic female mice can be housed together in the automated home-cage imaging system. Mice are identified by RFID and wide-field, mesoscopic imaging of the dorsal cortex is performed to assess functional connectivity and responses to sensory stimuli. Here we show data from a cohort of mice which we monitored up to 24 hours a day over ~90 days. They initiated >7,000 imaging trials without the intervention of the experimenter. We have based the automated home-cage hardware on the Raspberry Pi single board computer. Mice tolerated 30 to 125 s sessions of head-fixation. Auto-head-fixed mice were able to produce maps of visually evoked responses (response to unilateral flash stimulus). The Pi controls the cage via a Python program which triggers LED illumination, a water reward valve and the solenoid driven head fixation mechanism while reading RFID tags and grabbing images from the camera module. Trials of head restraint are chosen at random and the user controls the percentage of head fixation via the Python software. Using the Pi minimizes cost and maximizes the potential to scale up the automated home-cage imaging to many vivarium hosted, remote controlled cages. The system is also ideal for scenarios where handling animals can perturb results such as studies of circadian rhythms, micro-biomes, or social interactions.



Disclosures: **J. Ledue:** None. **F. Bolanos:** None. **M. Vanni:** None. **G. Silasi:** None. **J. Boyd:** None. **D. Haupt:** None. **T.H. Murphy:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

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Topic: I.04. Physiological Methods

Support: The smart IT convergence system research center funded by the ministry of education, science and technology as global frontier project (CISS-2012M3A6A6054204)

The National Research Foundation of Korea(NRF) Grant 2014R1A1A1A05003770

The National Research Foundation of Korea(NRF) Grant 2014R1A2A2A09052449

Title: Neuronal activity depending on zinc concentration on multi-electrode array

Authors: ***H. JEONG**[1], S. HWANG[1], S. JUN[1,2];
[1]Electronics Engin., Ewha Womans Univ., Seoul-City, Korea, Republic of; [2]Brain and Cognitive Sci., Ewha Womans Univ., Seoul-city, Korea, Republic of

Abstract: Zinc is an essential trace element for mammalian cells and used as a neurotransmitter or modulator in the central nervous system. The highest concentrations in the brain were found in the hippocampus and the cerebral cortex where Zn^{2+} is primarily localized in nerve endings of glutamatergic neurons. It is reported that the exposure of neurons to high concentrated zinc leads to selective neuronal death. Moreover, zinc contribute to the brain function and neuropathology associated with a neuronal problem, such as Alzheimer's disease, stroke, and seizures. Nevertheless, exact function of zinc is still not well known. In this study, we observed the alteration of neuronal activity in accordance with the change of zinc concentration on the hippocampal neurons cultured on microelectrode arrays (MEAs). The zinc chloride was added exogenously into the cultured hippocampal neurons and concentration of zinc used in the experiments varied from 1uM to 100uM. Depending on the zinc concentration, the different phenomenon has been found. When the measurement was held by electrical method, the frequency of action potentials increased at low concentrations of zinc and decreased at high concentration. In addition, we measured the long-term time dependent changes of action potential activity at constant zinc concentration. The result showed that the effect was different according to zinc concentration.

Disclosures: **H. Jeong:** None. **S. Hwang:** None. **S. Jun:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.02/NNN17

Topic: I.04. Physiological Methods

Support: NIH Grant R43NS086118

Title: From lab-to-marketplace: Commercialization of a stretchable microelectrode array

Authors: *O. GRAUDEJUS[1], R. PONCE WONG[2], S. AHUJA[3], S. WAGNER[4], B. MORRISON, III[3];
[1]Sch. of Mol. Sci., BMSEED Llc/Arizona State Univ., Tempe, AZ; [2]BMSEED Llc, Tempe, AZ; [3]Biomed. Engin., Columbia Univ., New York, NY; [4]Electrical Engin., Princeton Univ., Princeton, NJ

Abstract: Most cells in the human body are constantly subjected to mechanical deformation, e.g., near the heart, the lung, and blood vessels. This physiological stretching and compressing is important for the proper functioning of cells, and is also an important cue for the differentiation of stem cells in tissue engineering applications. Stretching or compressing cells too much (pathological stretching) causes a trauma, e.g., traumatic brain injury (TBI) and spinal cord injury (SCI). It is currently not possible for *in vitro* models of TBI and SCI, nor in tissue engineering, to carry out electrophysiological measurements while stretching the cells. BMSEED's **M**icro**E**lectrode **A**rray **S**tretching **S**timulating **u**nd **R**ecording **E**quipment (MEASSuRE) enables such measurements, because the microelectrodes stretch and relax elastically with the cells. MEASSuRE consists of five modules: (1) the *s*tretchable microelectrode array (sMEA) contains at its center the microelectrodes that interface with the cell culture, and at its perimeter the interface with a data acquisition system; (2) the mechanical stretcher that applies strain to the culture under study; (3) software that controls the motion of the stretcher and its interface with the data acquisition system; (4) an optional microscope with video camera captures images of the cells before, during and after stretching; (5) a data acquisition system for amplification, storage, and manipulation of the recorded data. MEASSuRE has the following demonstrated capabilities: (i) stable mechanical and electrical interface with cell cultures during mechanical stretching, (ii) accurate comparison of pre- and post-stretch responses, (iii) high reproducibility of mechanical strain, (iv) biocompatibility, and (v) repeated stretch and relaxation enabling the study of the accumulated effect of repeated blows to the head that individually do not cause injury (soldiers, American football players). In previous work, we have demonstrated the utility of sMEAs in neurotrauma research. sMEAs were critical in the investigation of the impact of TBI on bicuculline-induced, long-lasting network synchronization in the hippocampus. The mechanical stimulation from TBI significantly disrupted bicuculline-

induced, long-lasting network synchronization (a measure of learning ability) 24 hours after injury, despite the continued ability of the injured neurons to produce action potentials (Kang et al., J. Neurotrauma, 2015). BMSEED LLC is currently developing the fabrication technology with funding from an NIH SBIR award to make MEASSuRE widely available commercially by providing an easy-to-use, integrated system.

Disclosures: O. Graudejus: None. **R. Ponce Wong:** None. **S. Ahuja:** None. **S. Wagner:** None. **B. Morrison:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

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Topic: I.04. Physiological Methods

Support: NSF GRFP 0940902

NSF IGERT 1250104

DARPA Young Faculty Award D14AP00049

Title: Immobilization induces a sleep-like state in *C. elegans*

Authors: *D. L. GONZALES[1,2], K. N. BADHIWALA[3], J. T. ROBINSON[2,3,4];
[1]Applied Physics, [2]Electrical and Computer Engin., [3]Bioengineering, Rice Univ., Houston, TX; [4]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Sleep is necessary for most animals, yet the neural circuits involved in this process are difficult to study in anatomically complex animals such as mice and primates. In the roundworm *C. elegans*, periods of lethargus provide a powerful model behavior for probing the molecular, genetic and neural mechanisms of sleep[1-3]. These quiescent states are hallmarked by locomotive cessation and reduced calcium activity; however, we have found no studies of electrophysiological activity during quiescence due to the invasive dissections necessary for patch-clamp measurements. To overcome this challenge we used our recently developed nanoscale suspended electrode arrays (nano-SPEARs) to measure body-wall muscle electrophysiology in intact *C. elegans* for extended periods of time. We found that worms confined to a narrow microfluidic channel show a substantial decrease in body-wall muscle activity resembling a sleep-like state. This quiescence appears in the absence of external heat or other noxious stimuli, therefore we hypothesize that worms are stressed by the mechanical forces presented by narrow microfluidic channels. To the best of our knowledge, this is the first

observation of quiescence in adult worms triggered by mechanical stimuli. To better understand this state we used simultaneous electrophysiological recordings from the body-wall muscles and neuronal calcium imaging to measure the effects of mechanosensor loss of function mutations on this quiescent state. Additionally, we attempt to dissect the neural circuit involved in this stress response by optogenetic manipulation of specific neurons known to be involved in mechanoreception and lethargus. Finally, we discuss the potential to use this state to study habituation and sensitization in immobilized *C. elegans*. 1. Cho J. Y. and Sternberg, P. W. (2014). Multilevel modulation of a sensory motor circuit during *C. elegans* sleep and arousal. *Cell*, 156, 249-260. 2. Hill A. J., Mansfield R., Lopez J. M. N. G, Raizen D. M. and Buskirk C. V. (2014). Cellular stress induces a protective sleep-like state in *C. elegans*. *Current Biology*, 1-7. 3. Raizen D. M., Zimmerman J. E., Maycock M. H., Ta U. D., You Y., Sundaram M. V, and Pack A. I. (2008). Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature*, 451, 569-72.

Disclosures: D.L. Gonzales: None. **K.N. Badhiwala:** None. **J.T. Robinson:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

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Program#/Poster#: 469.04/NNN19

Topic: I.04. Physiological Methods

Support: University of Oslo

Research Council of Norway Grant No. 216699

Title: A novel electrodiffusive scheme for modeling ion dynamics in neural tissue

Authors: *A. V. SOLBRÅ[1], A. MALTHE-SØRENSSEN[1], G. T. EINEVOLL[2,1], G. HALNES[2]; [1]Dept. of Physics, Univ. of Oslo, Oslo, Norway; [2]Norwegian Univ. Life Sci., Aas, Norway

Abstract: In models of brain dynamics, most focus has been put on the electrical dynamics of neurons. A common modelling assumption is that the extracellular space represents an isopotential background where ion concentrations remain constant over time. However, periods of neural hyperactivity may induce local shifts in extracellular space ion concentrations, and such shifts are associated with pathological conditions such as hypoxia, anoxia, ischemia and spreading depression.

Ion dynamics in the extracellular space depends on both diffusion and electric drift. Previous electrodiffusive models of extracellular space dynamics have typically been based on the Poisson-Nernst-Planck (PNP) formalism, which computes the ion dynamics from the Nernst-

Planck equation, and the electric field from the Poisson equation. The PNP formalism is highly accurate, and explicitly accounts for charge relaxation processes that take place on very small temporal and spatial scales (nanoseconds and nanometers). Accordingly, the PNP formalism is extremely computationally expensive, and unsuited for simulations at the tissue level.
An alternative to the PNP scheme is the electroneutral scheme, which replaces Poisson's equation with the constraint of local electroneutrality (Mori et al, PNAS, 2008). Recently, we developed the Kirchhoff-Nernst-Planck (KNP) scheme, a version of the electroneutral scheme which was particularly tailored to simulate extracellular space dynamics at the tissue level (Halnes et al, Plos Comp Biol, 2013; Arxiv, 2015). This scheme does not model the charge relaxation processes explicitly; instead it ensures that no net charge enters a subvolume of the bulk solution at any time point in the simulation, which is approximately true at time scales larger than a few nanoseconds.
Here, we compare the performance of the KNP and PNP schemes in terms of accuracy and computational efficiency in various scenarios, and identify the conditions under which the KNP scheme represents a useful approximation. For simulations at the level of neural tissue, we show that the KNP scheme allows for a dramatic computational speed-up, with very little loss in accuracy in the predicted dynamics of ion concentrations and the electrical field in the extracellular space at timescales longer than microseconds.

Disclosures: **A.V. Solbrå:** None. **A. Malthe-Sørenssen:** None. **G.T. Einevoll:** None. **G. Halnes:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.05/NNN20

Topic: I.04. Physiological Methods

Support: ARC Future Fellowship FT120100619

Discovery Project DP130100194

NHMRC Project APP1103923

Title: Local versus global effects of isoflurane anesthesia on visual processing in the fly brain

Authors: ***D. COHEN**[1], O. H. ZALUCKI[2], B. VAN SWINDEREN[2], N. TSUCHIYA[1]; [1]Psychology, Monash, Melbourne, Australia; [2]Queensland Brain Inst., Brisbane, Australia

Abstract: What characteristics of neural activity distinguish the awake and anesthetized brain? Drugs such as isoflurane abolish behavioral responsiveness in all animals, implying evolutionarily conserved mechanisms. However, it is unclear whether this conservation is reflected at the level of neural activity. Studies in humans have shown that anesthesia is characterized by spatially distinct spectral and coherence signatures that have also been implicated in the global impairment of cortical communication. We questioned whether anesthesia has similar effects on global and local neural processing in one of the smallest brains, that of the fruit fly (*Drosophila melanogaster*). Using a recently developed multi-electrode technique, we recorded Local Field Potentials (LFPs) from different areas of the fly brain simultaneously, while manipulating the concentrations of isoflurane. Flickering visual stimuli ('frequency tags') allowed us to track evoked responses in the frequency domain and measure the effects of isoflurane throughout the brain. We found that isoflurane reduced power and coherence at the tagging frequency (13 or 17Hz) in central brain regions. Unexpectedly, isoflurane increased power and coherence at twice-the tag frequency (26 or 34Hz) in the fly's optic lobes, but only for specific stimulus configurations. By modeling the periodic responses, we show that the increase in power in peripheral areas can be attributed to local neuroanatomy. We further show that the effects on coherence can be explained by impacted Signal to Noise Ratios (SNR). Together, our results show that general anesthesia has distinct local and global effects on neuronal processing in the fruit fly brain.

Disclosures: D. Cohen: None. **O.H. Zalucki:** None. **B. van Swinderen:** None. **N. Tsuchiya:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.06/NNN21

Topic: I.04. Physiological Methods

Title: Cognitive and symptom correlates of EEG time-frequency domain decomposition during a flanker task in subjects with PTSD

Authors: *G. MAY[1,2,3], H. WAHBEH[4], S. NELSON[1,2,5];
[1]VA VISN 17 Ctr. of Excellence, Waco, TX; [2]Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX; [3]Psychiatry and Behavioral Sci., Texas A & M Univ., Temple, TX; [4]Oregon Hlth. & Sci. Univ., Portland, OR; [5]Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: PTSD remains a difficult disorder to treat, in part because it is suspected to comprise homogenous subtypes that respond differentially to treatment. EEG event-related spectral

dynamics provide a time-varying measure of oscillatory brain activity, and may serve as a set of biomarkers by which patients can be clustered. They have been used to discriminate subjects with severe PTSD from healthy controls, but to our knowledge, they have not been used to identify homogeneous clusters of patients with PTSD. 64 patients with a mean age 50.3 years completed a number of psychological measures, and underwent a 10 minute flanker task. Evoked potentials were decomposed to the time-frequency domain using wavelet convolution. Subjects were clustered using UPGMA, yielding a cophenetic correlation coefficient of 0.86. Clusters were generally laid out along a single principle component axis, which weakly correlated with several indicators of mental health as well as adherence to therapy. This dataset demonstrates feasibility of clustering patients based on the time-frequency decomposition of a task-related EEG. Future work might focus on stimuli that are designed to elicit differential responses in putative subtypes of PTSD, and use fMRI to elucidate the anatomical sources of functional differences between groups.

Disclosures: G. May: None. **H. Wahbeh:** None. **S. Nelson:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.07/NNN22

Topic: I.04. Physiological Methods

Support: HBP Grant 604102

Title: Biophysical modeling of single-neuron contributions to EEG and ECoG signals

Authors: *S. NAESS[1], T. V. NESS[2], G. HALNES[2], E. HALGREN[3], A. M. DALE[3], G. T. EINEVOLL[2,1];
[1]Univ. of Oslo, Oslo, Norway; [2]Norwegian Univ. of Life Sci., Aas, Norway; [3]UCSD, San Diego, CA

Abstract: Electroencephalography (EEG), i.e., recordings of electrical potentials at the scalp, and electrocorticography (ECoG), i.e., potentials recorded on the cortical surface, are two prominent techniques probing brain activity at the systems level. Despite their long history and widespread use, the proper interpretation of these brain signals in terms of the biophysical activity in underlying neurons (nerve cells) and neuronal networks is still lacking. Present-day analysis is predominantly statistical and limited to identification of phenomenological signal generators without a clear biophysical interpretation. New biophysics-based analysis methods are thus needed to take full advantage of these brain-imaging techniques (Einevoll et al., Nat Rev

Neurosci, 2013).
Here we used biophysical modeling based on morphologically detailed multicompartmental neuron models to explore single-neuron contributions to ECoG and EEG signals and in particular the feasibility of using the so-called current-dipole approximation in predicting these signals (Hamalainen et al, Rev Mod Phys, 1993). Specifically, we used the open-source Python package LFPy (lfpy.github.io) which builds on Neuron (www.neuron.yale.edu) and is based on well-established volume-conductor theory for numerical calculations of extracellular potentials. The LFPy package was supplemented with new Python tools for calculating the current-dipole moment of a neuron for use of the current-dipole approximation to predict ECoG and EEG signals. Current-dipole approximations were explored in the inhomogeneous four-concentric-spheres head model (Srinivasan et al., IEEE Trans Biomed Eng, 1998), and compared with results from using the Finite Element Method (Dhatt et al., John Wiley & Sons, 1977).
When comparing computed cortical-cell contributions to the EEG and ECoG signals from using the current-dipole approximation with results from the full model explicitly including all transmembrane currents, we find that the current-dipole approximation is applicable for modeling EEG signals. This allows for a drastic simplification of future biophysics-based computation of EEG signals from cortical cell populations. However, we find that the current-dipole approximation is not generally applicable for computing ECoG signals.

Disclosures: **S. Naess:** None. **T.V. Ness:** None. **G. Halnes:** None. **E. Halgren:** None. **A.M. Dale:** None. **G.T. Einevoll:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.08/NNN23

Topic: I.04. Physiological Methods

Title: A library of human electrocorticographic data and analyses

Authors: *K. J. MILLER[1], J. OJEMANN[2];
[1]Neurosurg., Stanford, Stanford, CA; [2]Univ. of Washington, Seattle, WA

Abstract: Electrophysiological data from implanted electrodes in humans are rare. Most recordings that have been performed are with epilepsy patients who have electrocorticographic (ECoG) electrodes implanted in the course of diagnostic localization of seizure focus prior to surgical resection. Only a small group of scientists have had the opportunity to work with these patients, and access to ECoG data has remained somewhat exclusive. It is recorded at only a few institutions around the country, often with different amplification setups, sampling rates, and

behavioral variations (even within the same institution).
Therefore, we have compiled a set of 16 benchmark experiments, with over 200 individual datasets made with the same amplifiers, at the same settings, with the same person interacting with the subject and performing the experiment. Depending on where the electrodes were placed for clinical indication, we performed experiments known to be associated with covered brain areas. In every case, electrode positions have been registered to brain anatomy. All data, anatomic, and analysis files (MATLAB code) are in a common, intuitive file structure. Every study/task has at least 4 subjects with confirmed task- modulated signal change in at least 1 electrode. Four of the sixteen experiments have not been published in any form.
In the course of analyzing these data, a large number of novel analysis techniques were developed. We are releasing our code base with the data, in such a way that all figures from published manuscripts describing these data can be directly reproduced. These data, along with behavioral parameterizations, anatomic localizations, and brain- surface renderings are now available for download worldwide, without restriction on use (other than proper citation).
The experiments/analyses contained in the library are:
• Baseline fixation
• Simple cue-based movement of hand&tongue
• Individual finger movements
• Joystick cursor tracking
• Hand Gestures
• Baseline fixation (with high sampling rate to fit power law form)
• Movement imagery (hand&tongue, with corresponding movement data included as well)
• One Dimensional Cursor control by imagery-based feedback (with corresponding movement and imagery data)
• Real time speech mapping from Noun reading & Verb generation
• N-back working memory task with pictures of houses
• Visual search task
• Basic Face-house picture presentation tasks
• Noise-masked face-house picture presentation tasks
• Baseline fixation (phase-amplitude coupling analysis)
• Mouse cursor tracking
• Noun reading & verb generation repeat runs, with multiple word lists

Disclosures: K.J. Miller: None. **J. Ojemann:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.09/NNN24

Topic: I.04. Physiological Methods

Support: IWT SBO110068

Title: High-density opto-electrical neural probe based on silicon nitride photonics for single neuron recording and stimulation

Authors: *L. HOFFMAN[1,2,3], S. LIBBRECHT[3], M. WELKENHUYSEN[1,4], A. ANDREI[1], V. BAEKELANDT[3], S. HAESLER[2], D. BRAEKEN[1];
[1]IMEC, Leuven, Belgium; [2]NERF, Leuven, Belgium; [3]KULeuven, Leuven, Belgium; [4]marleen.welkenhuysen@imec.be, ;, Belgium

Abstract: We present a multichannel optoelectrical depth probe which enables low noise extracellular electrical recording and optogenetic manipulation of neural tissue with high spatio-temporal precision. Simultaneous recording and site-specific optogenetic manipulation are widely used to determine the contribution of particular neural populations to behavior. Moreover, it has become common practice to optically identify specific ChR2-tagged cell types in extracellular recordings. However, current technologies for combined optical and electrical brain interfacing are often bulky or only provide a limited number of optical/electrical channels. Improving this situation is the main goal of our optogenetic neural probe.

The probe features 24 low-impedance titanium nitride contacts alongside 12 optical outputs (‘optrodes'). These optrodes are activated with light carried through silicon nitride waveguides that originate at the base of the probe and run along the shank. At the optrode site, the light is extracted from the waveguide with an optical grating coupler which redirects the optical power almost perpendicularly away from the probe plane. Additionally, the probe has the highest number of combined electrodes and optrodes (n=36) reported so far with a small cross-sectional area (100 μm width and 30 μm thickness). The electrode and photonic platforms have been monolithically integrated with a fully CMOS compatible process in a semi-industrial pilot line.

Different options can be used to couple light into the proposed optical neuro probe, which are either based on laser diodes, optical fibers or LEDs. All of them have complementary advantages that can serve different applications, thereby increasing the versatility of the system. Finally, we present a characterization of these different options along with in vivo experiments, which demonstrate highly localized artefact free optical stimulation.

Disclosures: **L. Hoffman:** None. **S. Libbrecht:** None. **M. Welkenhuysen:** None. **A. Andrei:** None. **V. Baekelandt:** None. **S. Haesler:** None. **D. Braeken:** None.

Poster

469. Electrode Arrays I

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Program#/Poster#: 469.10/NNN25

Topic: I.04. Physiological Methods

Support: UCSD Frontiers of Innovation Scholars Program

Center for Brain Activity Mapping

Qualcomm Institute Calit2 Strategic Research Opportunities

University of California Office of the President Multicampus Research Programs and Initiatives

ONR N00014-13- 1-0672

Title: Spatial correlation in a 400 micron pitch electrocorticography grid

Authors: ***N. ROGERS**[1], J. HERMIZ[2], E. KAESTNER[3], M. GANJI[2], B. S. CARTER[3], S. S. CASH[4], D. BARBA[3], S. DAYEH[2], E. HALGREN[3], V. GILJA[2];
[1]Physics, [2]Electrical and Computer Engin., [3]Univ. of California San Diego, La Jolla, CA;
[4]Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Higher density electrocorticography (ECoG) grids allow for recordings with greater spatial resolution. In addition to recording from more locations on a relevant patch of cortex, higher density recording may open up the potential for new techniques for analyzing ECoG data. Features that are sensitive to the activity of smaller neural populations may become accessible at this scale. Here we explore the correlation between pairs of channels on the grid as a function of distance. Changes in the structure of this relationship measured at the sub-millimeter scale could be indicative of changes in the dynamics of underlying physiological responses.
The electrodes used were nanofabricated gold embedded parylene C with 40 um diameter contacts coated in PEDOT:PSS. The low impedance of PEDOT allows the contacts to be made smaller and with higher density while maintaining a tolerable signal-to-noise ratio. The electrodes were configured in a 7x8 grid with a pitch of 400 μm. For comparison, a standard clinical ECoG grid has contact areas three orders of magnitude larger, and a pitch that is over 20 times larger.

Correlation between channels gives a measure of the similarity between the signals measured, but it will also include external noise that appears on all channels. The average correlation between pairs of channels decreases with distance, and the rate at which it decreases depends on the frequency-- with lower frequencies generally maintaining a higher correlation across the grid. For pairs spaced at the pitch of the device, 400μm, the correlation across all frequencies is very high, 0.8 or higher. Between pairs of electrodes spaced at greater distances there is a smooth decrease of correlation with distance across theta (3-8 Hz), beta (15-30 Hz), gamma (30-50 Hz), and high-gamma (70-110 Hz). In an example experiment for pairs 2mm apart the high gamma bands will become mostly uncorrelated (correlation coefficient ~0.4) while the lower bands will maintain some correlation (0.5-0.8). These values are averages over tens of minutes of recording. However, the drop in correlation with distance changes on short time scales (on the order of seconds or faster).

Disclosures: **N. Rogers:** None. **J. Hermiz:** None. **E. Kaestner:** None. **M. Ganji:** None. **B.S. Carter:** None. **S.S. Cash:** None. **D. Barba:** None. **S. Dayeh:** None. **E. Halgren:** None. **V. Gilja:** None.

Poster

469. Electrode Arrays I

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Program#/Poster#: 469.11/NNN26

Topic: I.04. Physiological Methods

Support: EU Horizon 2020, grant agreement no. 644732

Title: Ear-EEG as a novel technology for wearable brain wave monitoring

Authors: *C. GRAVERSEN[1], E. B. PETERSEN[1,2], A. FAVRE-FELIX[1,3], L. FIEDLER[4], J. OBLESER[4], T. LUNNER[1,2];
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Abstract: Electroencephalography (EEG) is a well established technology widely used in brain research and clinical applications. Current technologies mainly record the potentials by multiple surface electrodes distributed over the scalp in a fixed laboratory environment. This approach generates potentials with high spatial and temporal resolution, which however is limited for every-day use due to stationary recording systems and visual design. To establish a platform feasible for every-day use in brain-computer-interface and clinical applications, we here propose

a wearable device recording the brain waves from the ear canal (Ear-EEG). Hence, the recordings are limited in spatial distribution, but on the other hand provide an invisible and easily wearable device that can be used 24/7. Furthermore, the sensors can even be integrated into a hearing aid, and by this approach be used to steer future hearing aids as a new type of brain-computer-interface for hearing-impaired persons. To test the optimal configuration of this new electrode array, several electrode materials, number of electrodes, positions, and references were tested. In our present design, we conducted a pilot study on 8 normal hearing persons by combined recordings from conventional 64 EEG scalp electrodes and three Ear-EEG electrodes in each ear. We found that the Ear-EEG in each ear was highly correlated to scalp-near electrodes as shown in figure 1. Furthermore, we demonstrated the possibility to record relevant neural responses from the Ear-EEG electrodes in both auditory oddball and audiobook paradigms. Additionally, we found that the neural responses assessed by cross-correlation between EEG responses and auditory stimuli statistical differed between auditory stimuli presented to the left ear compared to the right ear at several time-points. In conclusion, we expect the development of Ear-EEG devices to be a major step towards an unobtrusive and easy-to-use alternative to conventional EEG systems, which may pave the road for new EEG applications in brain-computer-interface and clinical applications.

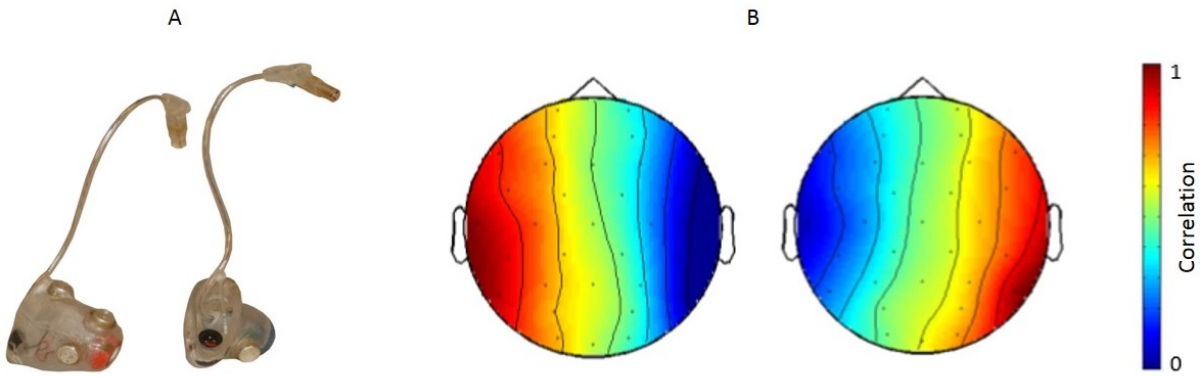

Figure 1. Ear-EEG devices with three electrodes in each earmold (A), and the correlation from the average Ear-EEG potentials in left and right ear respectively to each of the 64 scalp potentials (B).

Disclosures: **C. Graversen:** A. Employment/Salary (full or part-time): Eriksholm Research Centre - part of Oticon. **E.B. Petersen:** A. Employment/Salary (full or part-time): Eriksholm Research Centre - part of Oticon. **A. Favre-Felix:** A. Employment/Salary (full or part-time): Eriksholm Research Centre - part of Oticon. **L. Fiedler:** None. **J. Obleser:** None. **T. Lunner:** A. Employment/Salary (full or part-time): Eriksholm Research Centre - part of Oticon.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.12/NNN27

Topic: D.06. Vision

Support: NIH Grant R01-EY019693

Swartz Foundation Postdoc Fellowship

Title: An attention model of binocular rivalry

Authors: *H.-H. LI[1], J. RANKIN[2], J. RINZEL[3], M. CARRASCO[4], D. J. HEEGER[4]; [1]Dept. of Psychology, [2]Ctr. for Neural Sci., [3]Ctr. for Neural Science; Courant Inst. of Mathematical Sci., [4]Dept. of Psychology; Ctr. for Neural Sci., New York Univ., New York City, NY

Abstract: Purpose: Previous models of binocular rivalry fail to explain the evidence that rivalry ceases when attention is diverted away from the stimuli (Zhang et al., 2011; Brascamp and Blake, 2012). We propose a computational model of rivalry to address how the dynamics of rivalry depends on attention.
Methods: In the model, the sensory representation is composed of 4 monocular neurons (both eyes, selective for two orthogonal orientations), 2 binocular-summation neurons that sum responses of the monocular neurons with the same orientation preference, and 4 binocular-opponency neurons that compute differences between the monocular neurons. In addition, two 2nd-layer neurons, selective for orthogonal orientations, are driven by the responses of the summation neurons (low-pass filtered over time), and provide orientation-selective feedback to the monocular neurons. Rivalry is driven by: (1) Slow adaptation in monocular & summation neurons. (2) Attention: Orientation-selective feedback from the 2nd-layer neurons, analogous to feature-based stimulus-driven attention, is active when observers attend to the stimuli but silent when attention is diverted. This feedback enhances the multiplicative gain of whichever orientation has stronger sensory responses at any moment, and suppresses the gain of the other orientation. (3) Mutual inhibition: The opponency neurons respond when there is conflicting information between the two eyes, and provide subtractive suppression to the monocular population with weaker responses.
Results: We simulated the neural responses for two stimuli (dichoptic gratings and monocular plaid) under focused and diverted attention. The model exhibited rivalry for attended dichoptic gratings, but not for the plaid or unattended stimuli, consistent with experimental results. In addition, the model exhibited two hallmarks of binocular rivalry: (1) When the two images were swapped between eyes repetitively and rapidly, the dominant percept either stayed with one eye or followed one orientation for a period of time, depending on the temporal characteristics of the

stimuli relative to the low-pass filtering in the 2nd-layer neurons. (2) The dominance duration changed with stimulus strength following Levelt's propositions. A bifurcation analysis allowed us to completely characterize the model, and to identify the parameter values for which the model's behavior was consistent with experimental results.
Conclusion: Attention affects rivalry by biasing the competition toward the dominant feature, thereby stabilizing the percept. The dynamics of binocular rivalry are determined by a combination of attention and mutual inhibition.

Disclosures: H. Li: None. **J. Rankin:** None. **J. Rinzel:** None. **M. Carrasco:** None. **D.J. Heeger:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.13/NNN28

Topic: I.04. Physiological Methods

Title: A new 3D in-vitro model for studying human sensory neurons

Authors: *E. GRAS LAVIGNE[1], N. PY[2], D. BUTTIGIEG[1], L. L'HOMME[1], F. MAGDINIER[2], R. STEINSCHNEIDER[1];
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Abstract: Numbers of peripheral neuropathy are due to an abnormal innervation of skin or to a dysfunction of sensory receptors. For example in the peripheral neuropathic pain occurring in diabetes, patients have abnormal sensory perception like hypersensitivity to hot, burning sensation··· Thus it is important to develop pharmacological topical formulations able to treat symptoms and improve the life's quality of patient. In this way, some TRPV1 topical agonists, such as capsaicin, have already been used to desensitize dysfunctional sensory receptors. To test other agonists or antagonists of nociceptors or receptors implied in the skin inflammation process, we developed a new model of reconstructed skin innervated with human sensory neurons derived from induced pluripotent stem cells (iPS cells). To complete our 3D model we propose to use multi electrode arrays (MEA) system to evaluate the functionality of sensory receptors. Once the model will be characterized and calibrated with normal reconstructed skin, it will be possible to mimic pathological diabetic skin cells for example, and to assess topical formulation designed to treat symptoms. First we established the proof of concept by demonstrating that it is possible to produce functional human sensory neurons derived from iPS cells. We demonstrated by immunocytochemistry that these cells express several sensory

receptors (TRPV1, TRPA1, PAR2, opioid receptors…) and that these receptors are functional (calcium mobilization assays on TRPV1 and TRPA1, neurite outgrowth assays with NGF). Secondly we demonstrated that it is possible to assess sensory neurons activity using MEA systems. To do that, we proceeded in two steps. In the first step we used well characterized rat neurons and in the second step we made the comparison with our human sensory neurons. Another part of this project was to innervate reconstructed skin with human sensory neurons and to characterize the type and number of the different sensory receptors in our 3D innervated model by immunohistochemistry. Our final goal is to place the 3D innervated model on MEA system, to register the electrophysiological activity of sensory neurons upon different stimuli applied on the skin, to discriminate the different receptor activated and to decrease the activity of nociceptors by using topically applied formulations.

Disclosures: **E. Gras Lavigne:** None. **N. Py:** None. **D. Buttigieg:** None. **L. L'Homme:** None. **F. Magdinier:** None. **R. Steinschneider:** None.

Poster

469. Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: ERC Advanced Grant 267351 "NeuroCMOS"

International Institute for Education Whitaker Foundation Postdoctoral Scholarship

Title: Tunnel culture systems on microelectrode arrays to measure electrical conduction in a controlled-network system

Authors: *S. GEISSLER, A. HIERLEMANN;
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Abstract: In an effort to advance the common neurobiological tool set, our lab has developed a high-density microelectrode array based on CMOS technology, featuring 26,400 recording sites. Such high resolution allows efficient investigation of electrical conduction with multiple measurement sites along a single axon. In this project, we will introduce microtunnels to this microelectrode array system to direct axonal growth along specific paths while minimizing noise from neuronal background activity during recording. Using standard SU-8 photolithograpy techniques, we developed 5 μm × 5 μm polydimethylsiloxane (PDMS) tunnels connecting two culture chambers. E18 primary rat cortical neurons were isolated and cultured on coverslips and

on microelectrode arrays to examine cell function and growth under the tunnels. Neurons were successfully cultured in these devices and axons grew across the 500 μm long tunnels. *The goal of this project is to develop a culture system to introduce multiple cell types onto the microelectrode array in a controlled network to examine the effects of cell-cell interaction on signal conduction.* To achieve this goal, we have met two milestones; (1) we cultured neurons on microelectrode arrays to examine signal to noise ratio and learn the techniques required for multi-neuron recording, and (2) we developed microfluidic tunnel systems and cultured neurons in this device on coverslips. Next, we will culture neurons with the tunnel systems on the microelectrode array and add oligodendrocytes to the second culture chamber to examine the changes in electrical conduction through the axons after myelination. This in vitro platform will allow researchers to examine demyelinating diseases, test pharmaceutical effects on neurons, and measure real-time changes in neuronal activity.

Disclosures: S. Geissler: None. **A. Hierlemann:** None.

Poster

469. Electrode Arrays I

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Program#/Poster#: 469.15/NNN30

Topic: I.04. Physiological Methods

Support: NINDS 1U01NS094375-01

McKnight Foundation

Title: Laser and oxygen plasma treated carbon fiber electrode array for the detection of electrophysiological and dopaminergic activity

Authors: *P. R. PATEL[1], P. POPOV[2], A. MOHEBI[2], D. G. D. EGERT[3], A. A. HAMID[2], K. NAJAFI[3], J. D. BERKE[2], B. J. ARAGONA[2], C. A. CHESTEK[1];
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Abstract: Monitoring the electrical and chemical activity from neighboring but distinct populations of neurons while maintaining a stable interface is crucial to better understanding neural dynamics and information processing. This holds particularly true for the nucleus accumbens, a substructure of the striatum, which is of importance for decision making. Moreover, there are subregions of the nucleus accumbens (core and shell) that have functionally distinct dopamine release characteristics. To date, sampling from these subregions with multiple

electrodes has been difficult due to the size of existing glass insulated carbon fiber electrodes. To overcome this issue we have developed a high density carbon fiber electrode array that can alternatingly monitor electrophysiological and dopaminergic activity between the two subregions.
An array was fabricated using a thin (t=50μm) flexible printed circuit board as the underlying substrate. The board has a long extension (l=9mm) that permits measures within both the core and shell. At the end of this extension five carbon fibers, at a pitch of ~200μm, were secured using silver epoxy. The fibers extended past the edge by 500μm and were insulated with parylene-c. The tips of the fibers were then laser etched to re-expose a carbon surface and treated with oxygen plasma to remove any remaining debris. The laser and oxygen plasma steps result in a low electrode impedance and eliminate the need to electrodeposit poly(3,4-ethylenedioxythiophene). To implant the array, first a small glass cannula was inserted just above the nucleus accumbens, then the array was inserted into the cannula, and cemented in place.
For one rat, electrophysiology measurements were taken, during which time unit activity was detected on 9 of 15 days. Average unit peak-to-peak amplitude across all channels and days was 90μV. In addition, on the first and last day, phasic surges in dopamine concentration (i.e. dopamine 'transients'; indicative of dopamine release from burst firing of dopamine neurons) were detected on one fiber using fast scan cyclic voltammetry after the administration of a cocaine and raclopride mixture through a venous port.
Taken together, these results point to the viability of this technology to sense both electrophysiological activity within the striatum and dopamine fluctuations within the striatum's extracellular volume using the same electrode. Thus, the array configuration of these electrodes allows for sampling of electrical and chemical activity from neighboring, but still distinct, neuronal populations. Increasing the electrode density and count will only continue to provide more information about population dynamics.

Disclosures: **P.R. Patel:** None. **P. Popov:** None. **A. Mohebi:** None. **D.G.D. Egert:** None. **A.A. Hamid:** None. **K. Najafi:** None. **J.D. Berke:** None. **B.J. Aragona:** None. **C.A. Chestek:** None.

Poster

469. Electrode Arrays I

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Program#/Poster#: 469.16/NNN31

Topic: I.04. Physiological Methods

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

The Commissioned Research of National Institute of Information and Communications Technology (NICT)

Title: Validation of high density flexible ECoG arrays: monkey somatosensory evoked potential analysis.

Authors: *T. KAIJU[1], K. DOI[1], M. YOKOTA[1], K. WATANABE[3], M. INOUE[3], H. ANDO[3], K. TAKAHASHI[4], F. YOSHIDA[2], M. HIRATA[2], T. SUZUKI[3];
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Abstract: Recently much attention has been focused on the electrocorticogram (ECoG) as a source signal especially for clinical BMIs because of its good balance of features: Less invasive than penetrating electrodes, and higher spatial resolution than that of the scalp EEG. Until recently clinical ECoG electrodes were generally designed and utilized for identifying epileptogenic focus, and a typical size of those electrodes was diameter of ~4 mm and an inter-electrode distance of ~10 mm. However, to achieve more precise and naturalistic control of external devices such as multi-joint prosthetic arms, it may be beneficial to increase the number and density of recording channels to obtain richer motor/sensory information. There have been several reports attempting to increase the number and density of electrodes. However, little is reported about the validity of the high density ECoG arrays.Here, we report our analysis of somatosensory evoked potentials (SEP) of two monkeys in order to evaluate our high density ECoG array. We fabricated a high-density flexible array with MEMS technology. A gold layer was sandwiched between two Parylene-C layers (10 μm each). A unit array consisted of 32 channels. The inter-electrode distance was 700 μm, and the recording site of each channel was 350 μm square. Next, we executed an acute experiments using two macaques. The central sulcus was opened carefully and fabricated arrays (96-128 ch) were placed over the posterior wall of the central sulcus and the post-central gyrus, which corresponded to the digit representations of areas 1 and 3b. Spatiotemporally high-resolution SEPs were recorded by giving electrical stimuli and mechanical vibratory stimuli to each digit. Stimulation of different digits evoked different patterns of response and spatial patterns were approximately accordant with somatosensory mapping reported in previous researches using a penetrating method. In offline decoding analysis, we predicted stimulated fingers and stimulation intensity from SEP waveforms using a support vector machine. Especially in electrical stimulation condition, high (~98%) prediction accuracy was achieved. Further analysis showed that increasing channel counts improved prediction accuracy. On the other hand, depending on composition of feature vectors, combinations of just a few good channels could achieve high prediction accuracy. These results showed an effectivity of our higher density electrode on high-precision sensory decoding or spatiotemporal visualization of ECoG signal pattern. It is expected that utilization of high-density ECoG electrodes makes brain-machine interface more reliable.

Disclosures: **T. Kaiju:** None. **K. Doi:** None. **M. Yokota:** None. **K. Watanabe:** None. **M. Inoue:** None. **H. Ando:** None. **K. Takahashi:** None. **F. Yoshida:** None. **M. Hirata:** None. **T. Suzuki:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.17/NNN32

Topic: I.04. Physiological Methods

Support: Brain/MINDS from AMED, JAPAN

Title: Potential of optogenetic neuromodulation and ecog electrodes for bi-directional brain machine interface

Authors: ***F. YOSHIDA**[1,2], T. ARAKI[3], S. YOSHIMOTO[3], T. UEMURA[3], T. KAIJU[5,4], T. SUZUKI[5], T. SEKITANI[3], M. HIRATA[2,1];
[1]Osaka Univ. Med. Sch., Suita, Japan; [2]Global Ctr. for Med. Engin. and Informatics, Osaka Univ., Suita, Japan; [3]The Inst. of Scientific and Industrial Res., Osaka Univ., Ibaraki, Japan; [4]Grad. Sch. of Frontier Biosci., Osaka Univ., Suita, Japan; [5]Ctr. for Information and Neural Networks, Natl. Inst. of Information and Communications Technol., Suita, Japan

Abstract: A brain-machine interface (BMI) is a device, which interfaces directly with brain to control an external efforter (e.g. a robotic arm or computer). Here we present an electrocorticogram (ECoG) -based BMI , utilizing a flexible ECoG electrode and optogenetic neuromodulation for sensory feedback. Position or touch sense is important for clinical applications of the BMI because ideal prosthetic limbs should be perceived as natural extensions of the users' bodies. We have started to design a cortical modulator using optogenetics- a new method for the manipulation of neurons to dial in potential sensory input in a bi-directional manner. Optogenetics is based on genetically modified ion channels that respond directly to light. These light-gated ion channels, such as Channelrhodopsin-2 (ChR2), allow precise, millisecond control of specific neurons. This technique reduces most of the key problems associated with electrical brain stimulation: there is no associated electrical artifact to interfere with the electrophysiological recordings, nor any tissue damage from the current injection. It also allows for precise control of the spatial pattern of stimulation. A prototype optogenetic implant is presented, that will simultaneously record the activity of cortical neuronal ensembles and bring complex modulation patterns through optogenetic stimulation of cortical sensory areas. Our newly-invented optogenetic devices consist of both flexible ECoG and LED for optical stimulation. Here we report data from initial bench testing and implantation for the flexible

ECoG with LED in both the rat and non-human primate. We have shown that the flexible ECoG is effective as a chronic implant in rats, providing high fidelity neural recordings for up to 7 weeks. The initial results suggest that the new ECoG array can be successfully translated from rodents to accommodate the technological challenges associated with successfully interfacing with the non-human primate brain.

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Poster

469. Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: NRF-2015R1A2A1A09003605

Title: Measurement and characterization of low frequency extracellular potential from cultured neuronal networks

Authors: S. JOO, *Y. NAM;
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Abstract: To study the signal processing such as learning and memory between neurons in network scale, in vitro neural network have been used as a powerful and controllable model system. Measuring spike from neural network means that spatial and temporal activity of each neuron in network. In the brain, the recording of local field potential (LFP) by extracellular recording methods enable us to infer the physiological activity of a population of neurons. Even if the analysis of LFP was important to understand the information transfer principles of the brain, no previous studies have examined the LFP in cultured neural networks. Here, we report the recordings of LFPs and spikes from in vitro neuronal circuits cultured on the microelectrode array (MEA). We tracked the feature of LFP signals during the development of the networks and confirm that the measured spontaneous LFP were originated from synaptic activity of the neural network.We cultured clustered neural network on 60-channel ITO MEA by fabricating agarose hydrogel microwells through the MIMIC (Micromolding in capillaries) technique. On the MEA, neurons formed the clusters which were around 100 micrometer in diameter on the electrode array due to the patterned agarose hydrogel. From the networks, electrical signals which were measured by amplifier with wideband filter (Gain: 50, bandwidth: 0.02 Hz ~ 8 kHz) contained both spiking activity with high frequency component and LFP with low frequency component.

From immature stage of the network, there were no spiking activities from the clustered neural network. On the other hand, LFP traces were recorded from the networks spontaneously. We measured features (power spectrum, amplitude, and half-maximum full width) of LFP signal. Using drug response test, we confirmed that the LFP type signals is derived from synaptic activity. In this study, we showed that LFP and spike from clustered neural network was successfully measured by MEA system. Our method can provide diversity about LFP parameter for studying of neural network dynamics with in vitro cultured neural network.

Disclosures: **S. Joo:** None. **Y. Nam:** None.

Poster

469. Electrode Arrays I

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UCSD Center for Brain Activity Mapping

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University of California Office of the President Multicampus Research Programs and Initiatives

ONR N00014-13- 1-0672

Title: Assessing very high density intraoperative ECoG grids using a 7x8 grid with 400 um pitch

Authors: ***J. HERMIZ**[1], N. ROGERS[1], E. KAESTNER[1], M. GANJI[1], B. CARTER[1], S. CASH[2], D. BARBA[1], S. DAYEH[1], E. HALGREN[1], V. GILJA[1];
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Abstract: There is growing interest in higher densities grids (>2 channels/cm^2) for human electrocorticography (ECoG). A key question raised by this trend is, “What is the optimal density?” Tradeoffs between design parameters need to be measured to address this question. In this work, we begin addressing this question by describing the design space and comparing decoding performance of “virtual” square grids constructed by sub-sampling a 7x8 grid with 400 um pitch and 40 um diameter electrodes. This allows assessment of grids with densities as high as 670 channels/cm^2.There are 3 design parameters for square grids (excluding electrode

geometry): channel count (c), pitch (p), and area (A). We search this design space (S) for all possible square grids, varying c and p, as there are only 2 degrees of freedom. To assess grid performance, we trained a linear model to decode trials (n=180) where the subject was presented with 3 classes of audio-visual stimuli intraoperatively. Spectrotemporal features, integrating band power relative to stimulus onset, were used: {(4-15),(15-30),(30-55),(70-115)} Hz × {(0-0.25),..,(1.25-1.50)} sec. We used an algorithm called Shrunken Centroids Regularized Discriminant Analysis to learn the model because it has been shown to have competitive performance for high-dimensional datasets with limited training data (Guo et al., Biostatistics 2007). The performance metric used is percentage of trials decoded accurately. As expected, optimal performance was achieved when all 7x8 channels were used: 73% +/-7% (95% CI s.e.m; baseline chance is 33%; 8-fold cross validation). Consistent with this result, we found that when fixing p = 400 um or p = 800 um, there was a significant correlation (ρ) between accuracy and channel count, ρ = 0.54 and 0.49, respectively ($P < 0.01$, t-test). It's important to observe that A also increases with c when p is fixed. To examine if higher density grids can be more useful than lower density grids, we restricted A and increased c to see if accuracy increased. A reasonable comparison would be for all virtual grids $s_h \in S$ s.t. c=25,p=400 um and $s_l \in S$ s.t. c=9,p=800 um. The distribution of performance change between high and low density grids is positively skewed with an 80% confidence interval ranging from -8.7% to +17%, the difference between the medians of these distributions is statistically significant at 4.5% ($P < 0.01$, Wilcoxon signed-rank test), indicating that s_h can be more informative than s_l.

Disclosures: **J. Hermiz:** None. **N. Rogers:** None. **E. Kaestner:** None. **M. Ganji:** None. **B. Carter:** None. **S. Cash:** None. **D. Barba:** None. **S. Dayeh:** None. **E. Halgren:** None. **V. Gilja:** None.

Poster

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Program#/Poster#: 469.20/NNN35

Topic: I.04. Physiological Methods

Support: NIH 1U01NS094190

Title: A 512-channels, whole array readout, CMOS implantable probe for acute recordings in the brain

Authors: ***M. MALERBA**[1], G. ANGOTZI[1], G. MANDELBAUM[2], B. SABATINI[2], L. BERDONDINI[1];
[1]Italian Inst. of Technol., Genova, Italy; [2]Harvard Med. Sch., Boston, MA

Abstract: Despite huge progress, the understanding of the mechanisms underlying brain complexity is still hindered by a lack of experimental methodologies, able to disentangle, at a single-neuron level, the largely distributed functional brain circuits. From a neuro-technological point of view and with the aim of providing new experimental capabilities, the aim of our work is to realize a novel generation of microelectronic micro-machined neural probes that can provide large-scale single-neuron recording capabilities in different brain circuits [1]. As already demonstrated in-vitro on brain slices, cell cultures and retina whole mounts [2], a highly integrated design based on CMOS technology enables whole array recordings at sub-millisecond resolution from densely packed microelectrodes, allowing to literally image spiking activity and field potentials in multiple brain circuits at the same time. Here, we report on a complete probe system designed for in-vivo recordings with scalable circuit architecture. In detail, we present a modular approach in which 16 matrices yield 512 electrode-pixels along a single shaft, realizing a first generation of implantable CMOS neural probes. The device is capable of recording from the whole electrode array at sub-millisecond resolution, over a sensing area of 6 mm in length and 110 μm in width. Each electrode is an active pixel that comprises the recording site and an underneath first-stage amplifier; each module counts 32 pixels (25μm pitch), a column buffer for time division multiplexed readout (30kSample/s per pixel) and a programmable gain amplifier for further signal amplification (42dB to 72dB). An active feedback loop, shared among multiple electrodes, finally allows the cancellation of DC offsets without the need of integrating large input capacitors. To minimize tissue damage upon insertion, silicon micromachining techniques were used to shape the probe and reduce overall dimensions to LxWxH = 6 mm x 110 μm x 50 μm. We will report on recording capabilities, validated both in-vitro from mouse brain slices and in-vivo from head-fixed behaving mice, also discussing perspectives and future development. The final goal is coupling such devices with state-of-the-art optogenetic stimulation tools.
1. Angotzi, G. N., et al. "A 512-channels, whole array readout, CMOS implantable probe for acute recordings from the brain." *Engineering in Medicine and Biology Society (EMBC), 2015 37th Annual International Conference of the IEEE*. IEEE, 2015.2. Maccione, A., et al. "Following the ontogeny of retinal waves: pan- retinal recordings of population dynamics in the neonatal mouse." The Journal of physiology 592.7 (2014): 1545-1563.

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Poster

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Champalimaud Foundation

Sainsbury Wellcome Centre

Title: Validating silicon polytrodes with paired juxtacellular recordings

Authors: *J. P. NETO[1,2,3], G. LOPES[2], J. FRAZÃO[2], J. NOGUEIRA[2], P. LACERDA[2], E. FORTUNATO[3], P. BAIÃO[3], A. AARTS[4], S. MUSA[5], A. ANDREI[5], P. BARQUINHA[3], A. KAMPFF[1,2];
[1]Kampff Lab., Sainsbury Wellcome Ctr., London, United Kingdom; [2]Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [3]CENIMAT I3N, Caparica, Portugal; [4]Atlas Neuroengineering, Leuven, Belgium; [5]IMEC, Leuven, Belgium

Abstract: Cross-validating new methods for recording neural activity is necessary to accurately interpret and compare the signals they measure. Here we describe a procedure for precisely aligning two probes for *in vivo* "paired-recordings" such that the spiking activity of a single neuron is monitored with both a dense extracellular silicon polytrode and a juxtacellular micro-pipette. Our new method allows for efficient, reliable, and automated guidance of both probes to the same neural structure with micron resolution. We also describe a new dataset of paired-recordings, which is available online. We propose that our novel targeting system, and ever expanding cross-validation dataset, will be vital to the development of new algorithms for automatically detecting/sorting single-units, characterizing new electrode materials/designs, and resolving nagging questions regarding the origin and nature of extracellular neural signals.

Disclosures: J.P. Neto: None. **G. Lopes:** None. **J. Frazão:** None. **J. Nogueira:** None. **P. Lacerda:** None. **E. Fortunato:** None. **P. Baião:** None. **A. Aarts:** None. **S. Musa:** None. **A. Andrei:** None. **P. Barquinha:** None. **A. Kampff:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 469.22/NNN37

Topic: I.04. Physiological Methods

Support: ONR N00014-13-1-0672

UCSD Center for Brain Activity Mapping

Title: Cognitive responses recorded during neurosurgery using microarray pedot:pss electrodes

Authors: *E. KAESTNER[1], J. HERMIZ[2], N. ROGERS[2], M. GANJI[2], R. CARTER[2], S. CASH[3], D. BARBA[2], S. DAYEH[2], V. GILJA[2], E. HALGREN[2];
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Abstract: Human intracranial recordings combine spatial and temporal precision unmatched in modern human neuroimaging. But these recordings are rare and spatial coverage is often coarse and dictated by clinical considerations. Our team developed an approach to address both these concerns. First, to increase the number of recordings we developed a system to record cognitive activity during awake neurosurgeries. Second, we have developed a poly(3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT:PSS) micro-array of 56 electrodes with high spatial precision (400 um pitch) able to be placed on the pial surface during surgery. Recordings: During awake neurosurgery for clinical language mapping, patients volunteered for a 10 minute task and microarray placement. The PEDOT:PSS microarray was placed on the anterior superior temporal gyrus (STG) for Patient 1 (P1) and posterior STG for Patient 2 (P2). P1 was asked to read visual words, repeat auditory words, and name visual pictures. P2 was asked to make a match/mismatch decision about paired letters and auditory phonemes. Interspersed were visual (false fonts) and auditory (noise-vocoded) control stimuli. Results: Analysis focused on high-gamma amplitude (HGA; 70-170Hz) and linear discriminant modeling (LDM). For HGA, ANOVAs were run between stimulus classes and corrected with false-discovery rate. For the LDM, we used Shrunken Centroids Regularized Discriminant Analysis. Task performance during surgery was high (P1 verbally responded on >95% of trials; P2 was correct on 98% of trials). P1 had 42 good electrodes (impedance < 60k ohms @ 1kHz) with 15 electrodes (36%) showing a HGA increase to auditory words relative to visual words and pictures. LDM discriminated the 3 language modalities at 70% accuracy (chance: 33%). P2 had 33 good electrodes with all showing a HGA increase to auditory noise trials relative to the other three trial types. With no response to visual stimuli at this electrode placement, LDM only discriminated the 4 trial types at 32% accuracy (chance: 25%) but was able to discriminate human voice from noise-vocoded stimuli at 76% (chance: 50%). Discussion: During surgery, cognitive responses can be recorded and discriminated from PEDOT:PSS micro-arrays. This approach promises to increase the number of recordings by expanding the volunteer pool to anyone undergoing awake neurosurgery. Second, the high-density PEDOT microarray can reliably discriminate stimuli, both between language modalities (P1) and within a language modality (P2). Combining these advantages will allow us to test theories of language function rapidly and with a high degree of anatomical precision.

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Poster

469. Electrode Arrays I

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Title: A long-term feasibility study for neural recording with carbon-fiber based microelectrode array

Authors: *Y. LEE[1], Y. LIM[2], S. HWANG[1], S. JUN[1,3];
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Abstract: Microelectrode arrays are commonly used to record the neural activities in the brain or to make a neuronal interface between brain and machine. However, the traditional system often induces tissue reaction around the implanted microelectrode array, thereby limiting the possibility of long-term experiments. It is believed that the extent of tissue damage is associated with the geometrical size of the electrode. In this study, in order to reduce the reactive tissue response, carbon fiber-based microelectrode arrays are developed. Since the diameter of the carbon fiber (7 μm in diameter) is much smaller than the conventional micro-wires, it is possible that the carbon fiber neural probe may be appropriate for the long-term neural recording. Here, we introduce a multi-channel microelectrode array where each channel is composed of carbon fiber bundles. To verify the feasibility of long-term recording, we implanted the carbon-fiber based electrode in the motor cortex of freely behaving rats and monitored movement related neural responses and impedances of electrode for several weeks. These were also compared with the electrophysiology data obtained from conventional tungsten electrode implanted either in the same or different animal. Finally, we conducted immunohistochemistry of brain tissues for two

different electrode systems to confirm the possibility of chronic implant for the new carbon-based electrode.

Disclosures: **Y. Lee:** None. **Y. Lim:** None. **S. Hwang:** None. **S. Jun:** None.

Poster

469. Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: 2014M3C7A1062894

HI12C-0113

Title: Persistence of dysfunctional auditory information processing following pharmacotherapy in Internet gaming disorder: an event-related potential study

Authors: ***M. PARK**[1], Y. KIM[1], J. LEE[1], D. KIM[2], J.-S. CHOI[1,3];
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Abstract: Internet gaming disorder (IGD), defined as inability to control internet-based games, leads to serious impairment in psychological and social functioning, but few studies exist that identify neurophysiological characteristics in IGD. The aim of this study was to determine neurophysiological markers associated with symptom changes in IGD patients following pharmacotherapy with outpatient management. Eighteen patients diagnosed with IGD (22.61 ± 5.10 years), and 29 healthy controls (HCs; 24.66 ± 3.80 years) participated in this study. IGD patients completed a 6-month of serotonin reuptake inhibitors (SRIs)-based pharmacotherapy. Event-related potential (ERP) were acquired during an auditory oddball task in participants who were young adult males. For the IGD patients, ERP recorded prior to and after treatment. Between-group differences and the pre-post treatment differences in P300 components were investigated using repeated measures analysis of variance, respectively. The primary treatment outcome was a change in score on the Young's Internet Addiction Test from before and after treatment. The IGD group showed significantly reduced P300 amplitudes at midline centro-parietal and parietal site compared with those in HC. Reduced P300 amplitudes in the IGD were not correlated with scores on Internet Addiction severity. After 6 months of treatment, there were no significant changes in P300 amplitudes between pre- and post-treatment of IGD, even though, the IGD patients exhibited significant improvements of their IGD symptoms measured by

Young's Internet Addiction Test. Furthermore, there were no significant ERP differences between responder and non-responder to a 6-month pharmacotherapy in patients with IGD. These results indicate that IGD has abnormalities of P300 index and reduced P300 amplitudes could be considered as a candidate trait marker of IGD. This study enhances our understanding of neurophysiological characteristics of IGD.

Disclosures: **M. Park:** None. **Y. Kim:** None. **J. Lee:** None. **D. Kim:** None. **J. Choi:** None.

Poster

469. Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: AMED Grant 15mk0104029h0202

Title: Trial for drug-induced epileptogenic phenotype classification in primary rodent neurons and human induced pluripotent stem cell- derived neurons

Authors: ***N. MIYAMOTO**[1], T. KADOWAKI[2], K. SAWADA[1];
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Abstract: Many drugs have been reported to have the risk of seizures. Spontaneous neuron activity recordings by multi-electrode array (MEA) system from networks of cultured neurons could be a good risk evaluation method for drug-induced seizure events. Spontaneous electrical activity in neural networks consists of action potential spikes and organized patterns of action potential bursts. Those activities are able to be observed after a couple of week's cultures in primary rat cortex and hippocampal neurons. Epileptogenic response was induced in those cells by $GABA_A$ antagonism with gabazine or picrotoxin as generation of periodic synchronized burst spike patterns among multiple electrodes in a probe. The response was enhanced in a dose-dependent manner of $GABA_A$ antagonism. We previously reported that the spontaneous neuron activity was hardly obtained from human induced pluripotent stem cell (hiPSC)-derived neurons alone; but we succeeded in accelerating the activity generation by co-culture with iCell neurons and mouse primary astrocyte conditioned medium (mACM). And the potentiated hiPSC-neurons by humoral factor(s) from mACM showed epileptogenic response pattern after gabazine or picrotoxin treatment as well as rodent primary cells. In this study, we developed new MEA data analysis application for classification of the epileptogenic phenotype by amplitude pattern in a synchronized burst event. The application digitalizes repetition number of strength change in the single burst event on selected threshold value of spike amplitude and period of time duration.

$GABA_A$ antagonism by gabazine and picrotoxin increased the number of repetition in a dose-dependent manner in both primary rodent neurons and hiPSC-neurons. Analysis of epileptogenic responses using reagents other than $GABA_A$ antagonists is underway. This application might be a useful tool for MEA data analysis for drug-induced epileptogenic phenotype classification. This research is supported by the grants for iPS Non-clinical Experiments for Nervous System (iNCENS) project in Research Grants on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development, AMED.

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Poster

469. Electrode Arrays I

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Title: Low intensity low frequency ultrasound neuromodulation in cultured rat hippocampal neuron

Authors: *S. HWANG[1], H. JEONG[1], S. KIM[1], Y. LEE[1], T.-S. KIM[3], S. JUN[1,2];
[2]Dept. of Brain and Cognitive Sci., [1]Ewha Womans Univ., Seoul, Korea, Republic of; [3]Kyung Hee Univ., Yongin, Korea, Republic of

Abstract: Ultrasound is non-invasive neuromodulation method with high spatial selectivity and high penetrating power. The advantage of noninvasiveness, ultrasound has gained increasing attention of promising treatment approach of neurologic disease. In the past few years, a number of studies have been performed to observe the affect to ultrasonic neurostimulation in a rodent model. However, the underlying mechanism of ultrasound induced neuro-modulation are sYTnot clearly elucidated yet. Therefore, we have been aiming to investigate the principles and verified the effective sonication time with low-intensity, low-frequency (LILFU) ultrasound. In this study, we used 0.5MHz center frequency ultrasound with 17.4 mW/cm^2 spatial-peak, time-

averaged intensity based on 0.12 MPa peak acoustic pressure, 600 Hz pulse repetition frequency and 320 cycle pulse duration. Primary hippocampal neurons were cultured from embryonic 17-day gestation Sprague Dawley rat and seeded in microelectrode array (MEA). At DIV 14, the medium was changed to artificial cerebral spinal fluid (aCSF) for 10 minutes and LILFU was applied to the neural networks. Individual spontaneous action potentials (APs) were analyzed before, during and after ultrasound application. The frequency of APs increased during and after ultrasound stimulation. In order to figure out the synaptic change induced by sonication, immunocytochemistry was performed.

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Poster

469. Electrode Arrays I

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Program#/Poster#: 469.27/NNN42

Topic: I.04. Physiological Methods

Support: W911NF-15-2-0054

Title: *In vivo* testing of the neural sewing machine: a method for inserting fine, flexible neural probes

Authors: ***T. L. HANSON**[1], C. DIAZ-BOTIA[2], S. JUNG[3], M. M. MAHARBIZ[3], P. N. SABES[1];
[1]Physiol., UCSF, San Francisco, CA; [2]Biomed. Engin., [3]EECS, Univ. of California, Berkeley, Berkeley, CA

Abstract: In an effort to improve the quality, bandwidth, and reach of neural interfaces, we have been working on a system of fine and flexible electrodes that are robotically implanted via a fine and maximally stiff needle - a neural 'sewing machine'. This integrated system is design to increase the range and targetability of large-scale neural interfaces and to improve recording longevity. Specifically, the system aims to alleviate problems due to mechanical impedance mismatch between the electrode and brain tissue by implanting flexible and very thin probes. The small probe size (4 um x 16um) was design to minimize the foreign body response. By inserting probes individually, they can be targeted to nearly any location and depth, while also avoiding vaculature, thereby reducing blood-brain barrier disruption.
The system has five tightly integrated components: 1) Platinum-polyimide electrodes, with a silicon carbide adhesion layer and parylene-SiO2 release layer; 2) Vacuum / hydrogen micro-

brazing chamber for fabricating stepped 12 x 25um tungsten-copper inserter needles; 3) 16-DOF inserter robot, including closed-loop critically-damped ballistic needle retraction, ultrasonic cleaning station, microdurotomy drill, and four high-resolution targeting microscopes; 4) Custom 65nm, 2.25mm x 2.25mm neuromodulatory integrated circuit, with 64 recording channels and 16 stimulation channels that is directly wirebonded to the electrodes; 5) suite of programs for closed-loop anatomical targeting of individual recording and stimulation sites.
We present extracellular recordings and histological responses from the full electrode-inserter system when 64-channel arrays were implanted both acutely and chronically in rats, as well as acutely in monkeys. Elements of scaling the system to a full 2048 chronically implanted channels in a monkey will also be discussed, particularly speed, targeting accuracy, and importantly overall system yield.

Disclosures: **T.L. Hanson:** None. **C. Diaz-Botia:** None. **S. Jung:** None. **M.M. Maharbiz:** None. **P.N. Sabes:** None.

Poster

469. Electrode Arrays I

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Program#/Poster#: 469.28/NNN43

Topic: I.04. Physiological Methods

Support: CRC-15-04-KIST

Title: Opto-EEG constructs mesoscopic and stimulant-dependent functional connectome in mice

Authors: ***S. LEE**[1], E. HWANG[2], D. LEE[3], W.-S. JUNG[4], J. CHOI[2];
[1]KIST, Seoul-City, Korea, Republic of; [2]KIST, Seoul, Korea, Republic of; [3]Inst. of Basic science, Daejeon, Korea, Republic of; [4]POSTECH, Pohang, Korea, Republic of

Abstract: Connectome, the comprehensive architecture of brain elements and connections among them (Sporns et al., PLoS Comput. Biol. 2005), is fundamental to the understanding of brain functions. Recently, technical advances in optical imaging allow us to construct structural connectome. However, major issues of functional connectome needed to be addressed with cell-specific, state dependent approach in diverse situation. However, it mainly has been discussed separately that macroscopic functional connectome and brain states because of lack of methods that satisfying both in freely behaving animal. Opto-EEG combines optogenetics and high density electroencephalogram (EEG) which is designed to obtain whole cortical activity with high spatial resolution (Lee et al., JoVE, 2011). Optrodes readily approach into the brain through the space between micro-electrodes of high density EEG. Opto-EEG makes it possible to

stimulate a cell-specific or region-specific target circuit and record the consequent cortical activation simultaneously. To visualize the dynamical changes of functional connectome, dynamical analysis and source localization (Lee et al., PLoS One. 2013) tools also have been developed. By applying the Opto-EEG to well-defined somatosensory circuit, we could demonstrate the difference between anatomical and functional connectome. Repetitive optogenetic stimulation of ventral posteromedial nucleus of thalamus induced cortical activation propagating from the primary somatosensory to other cortices. Different propagation patterns were observed with respect to the stimulation frequencies, such that the stimulation at beta frequency induced resonance of synchrony between somatosensory and motor cortex. On the other hands, stimulation at gamma induced rapid synchronization between both hemispheres whereas the stimulation at lower frequencies showed gradually propagating pattern. This demonstration shows that different functional outcomes arise from a single entity of somatosensory circuit, depending on the diverse stimulation conditions. This result suggests that differently anatomical connectome, functional connectome is altered by stimulus conditions and opto-EEG becomes important piece to macroscopic functional connectome.

Disclosures: **S. Lee:** None. **E. Hwang:** None. **D. Lee:** None. **W. Jung:** None. **J. Choi:** None.

Poster

469. Electrode Arrays I

Location: Halls B-H

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Program#/Poster#: 469.29/NNN44

Topic: I.04. Physiological Methods

Title: Cortical spreading depression simulator

Authors: ***S. C. JONES**[1], Z. A. BARNES[2], A. M. BECK[2], W. S. HARLAM[2], B. J. RHINDRESS[2];
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Abstract: To aid in the development of a system to non-invasively detect cortical spreading depression (CSD), a CSD simulator consisting of a mechanical-electrical device that simulates the scalp surface voltage of a brain surface CSD, CerebroSim, has been designed and produced. This CSD simulator will enable pre-clinical bench testing of a non-invasive CSD detector to detect real-time scalp potentials in human patients.
CerebroSim generates a simulated CSD that propagates through electrically modeled layers of head tissue. The system consists of a touch screen user interface enabling: 1) the choice of CSD speed and various CSD propagation patterns, 2) the display of user details of the simulation, 3) a real-time display of the CSD motion, and 4) pausing or stopping the propagation at any time. The

electrical subunit of the device consists of custom demultiplexing printed circuit boards (PCBs) with integrated shift registers and transmission gates that control each pin of an 800 pin array. A Raspberry Pi microcontroller determines which pins output the -20 mV signal that is characteristic of a CSD. Pins not propagating the CSD signal act as disconnected and short circuit with a surrounding, aluminum ground plate, which is connected to a 20 mV, high output impedance signal. The plate prevents current draw into the array. With the ground plate maintained at 20 mV, the CSD signal propagated is held at 0 V to create the -20 mV differential. The pins are in contact with layered resistive fabrics that simulate the electrical properties of the cerebral spinal fluid, dura mater, skull, and scalp. The materials fit between two acrylic plates that "sandwich" layers to maximize contact and prevent expansion. The prototype CSD detection system will be validated by placing it on the topmost material layer (representing the skin) to locate and detect the simulated CSD propagation on the scalp surface.
With respect to circuit design, the simulator can generate complex CSD patterns of known forms. The system's ability to accurately control -20 mV transmissions to each pin of the electrode array was proven by direct measurement. Measured surface resistivity, multi-level isotropy, and unhindered voltage propagation of the layered resistive fabrics were found to be as expected. This physical CSD simulator will provide bench-testing and preliminary validation for a to-be-developed non-invasive CSD detection system before human clinical trials by providing a non-human model that simulates the scalp surface voltage of a brain surface CSD.

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Poster

469. Electrode Arrays I

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Title: Mapping and modeling EEG signals before and after a craniotomy procedure

Authors: *D. ISSAR[1], A. SNYDER[3], M. SMITH[2];
[1]Bioengineering, [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Electroencephalography (EEG) at the scalp is used to enhance our understanding of the brain's underlying computational mechanisms and for clinical diagnosis. It is the primary method for measuring electrical correlates of brain activity in humans because it is non-invasive; however, the signals weaken as they pass through layers of tissue, bone, and skin. With non-human subjects, experimenters use more precise but invasive methods of neural recording that penetrate the brain and require creating a small opening in the skull (craniotomy). EEG signals measured from animal subjects allow us to translate between internal brain signals observed through more invasive means and external brain signals that can be measured in human studies. However, the opening in the skull may alter the way the brain's electrical signals travel. We analyzed EEG signals from rhesus macaque monkeys during a visual attention task before and after perforating the skull to determine the effect on our method of mapping between neural activity and EEG signals. Using MRIs, we constructed pre- and post-craniotomy computer models of the electrical properties of each subject's head to infer the neural sources of the EEG signals. We analyzed the similarity between the pre- and post-models' time-courses, scalp electrical maps, and source localizations and found strong correlations regardless of whether there was a hole in the skull. These results suggest a craniotomy over visual cortex does not cause significant deviations in our ability to map visually evoked EEG signals. Our results are the first step in improving the translational ability between human and animal brain studies. This method of mapping EEG in the presence of a small hole in the skull is also important for the feedback and control capabilities of neural prosthetic devices that are often implanted via craniotomy.

Disclosures: **D. Issar:** None. **A. Snyder:** None. **M. Smith:** None.

Poster

470. Computational Tools for Circuit Mapping

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Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.01/NNN46

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

Support: Brain/MINDs Project

Title: Computational infrastructure to enable whole-brain mesoscale circuit mapping for Marmoset

Authors: *M. LIN[1], Y. S. TAKAHASHI[1], K. WEBER[1], K. HOSSAIN[1], B. HUO[1], A. S. TOLPYGO2[2], D. D. FERRANTE[2], S. BAI[3], M. G. ROSA[3], H. OKANO[1,4], P. P. MITRA[1,2]; [1]Brain Sci. Inst., Riken, Japan, Wakoshi, Japan; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Dept. of Physiol., Monash Univ., Clayton, Victoria, Australia; [4]Dept. of Physiol., Keio Univ. Sch. of Med., Shinjuku, Tokyo, Japan

Abstract: Modern whole-brain imaging techniques employing light microscopy generate large data volumes, requiring the development of specialized software tools to support the acquisition, quality control, pre-processing, analysis, and dissemination of such data sets. A software suite developed to this end in the Mouse Brain Architecture Project at CSHL, has been replicated and suitably modified to serve the needs of a high-throughput neurohistological pipeline at the RIKEN Brain Sciences Institute to process marmoset brains. The goal of the RIKEN BSI pipeline is to carry out brain-wide mesoscale circuit mapping of the common marmoset (*Callithrix Jacchus*) using the injections of anterograde and retrograde tracers, as part of the Japan Brain/MINDs project. The software suite developed includes a storage/communications setup, an integrated Laboratory Information Management System (LIMS), imaging quality control (QC), section registration and a web portal component, and is tailored to the scanning of whole marmoset brains at the light microscopy resolution together with auxiliary MRI scans of the same brains. The hardware infrastructure on which the suite is deployed uses a 10g network to connect image acquisition, storage and analysis engines. The image database consists of a distributed file system containing raw and compressed images in JPEG2000 format together with MySQL databases of metadata. A customized LIMS is used to track brains and associated metadata and also to facilitate laboratory process management. A responsive web data service is used locally for QC, and an IIPImage based viewer is used for remote web-based viewing of the high-resolution images for purposes of analysis and quality control. The neurohistological pipeline uses tape-transfer assisted cryosectioning that produces sections that preserve their rigid 2D geometries, but these need to be registered together to obtain 3D brain volumes. The corresponding section-registration code from the mouse pipeline was customized for the Marmoset, and is able to post-process the acquired images within a time frame consistent with the data acquisition rates, enabling a steady production of 3D brain volumes (~10TB/brain for LM data). Lessons learned from scaling up the mouse pipeline software to enable the steady processing of much larger marmoset brains (8x by volume) will be presented, together with the details of the associated software/hardware suite, and its integration with the associated web-portal for data dissemination. The successful replication and scaling up of the MBAP software pipeline demonstrates the generalizability of the approach to other species with brains of comparable sizes.

Disclosures: M. Lin: None. **Y.S. Takahashi:** None. **K. Weber:** None. **K. Hossain:** None. **B. Huo:** None. **A.S. Tolpygo2:** None. **D.D. Ferrante:** None. **S. Bai:** None. **M.G. Rosa:** None. **H. Okano:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

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Support: NIH TR01 MH087988

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NIH MH105949

NIH MH105971

NSF INSPIRE

NSF EAGER

Title: Computational topology algorithms for skeletonizing whole mouse brain tracer-injection data using discrete Morse theory.

Authors: *D. D. FERRANTE[1], S. WANG[2], Y. WANG[2], P. P. MITRA[1];
[1]Cold Spring Harbor Labs., Cold Spring Harbor, NY; [2]Computer Sci. and Engin., Ohio State Univ., Columbus, OH

Abstract: We digitized anterograde fluorescent tracer injected mouse brain sections and examined their skeletonization structure. Most existing neuron tracing algorithms require good neuron segmentation as preprocessing before the skeletonization step, and decisions in the segmentation step are typically based on local information, making it hard to capture the global structures behind data, and make the results noise-sensitive. Furthermore, the tracing methods often use greedy procedures to extract a neuron tree (e.g. approaches based on shortest path or maximum spanning tree), and the topology of the final tree relies heavily on the specific greedy strategies used. Non-homogeneous distribution of signal in input raw data remains challenging to handle, an issue that is especially important for tracer injection data, in which signal strength can differ greatly between the injection site to terminal regions. Finally, existing algorithms focus on single-neuron reconstruction, and do not address the more challenging problem of summarizing tracer injection data, which involves multiple neurons. We use recently developed techniques drawn from discrete Morse theory [cf. S. Wang et al., SIGSPATIAL/GIS 2015, DOI: 10.1145/2820783.2820833] to summarize tracer-injected whole-brain data sets. The output of our approach is a skeletonization of the tracer injection-label into a consensus tree structure. Our

Morse theory-based approach is effective at recovering the global one-dimensional non-linear (i.e., geometric graph) structure behind the input data and is robust without any special processing. No explicit segmentation or thresholding is needed. As our method relies on global information, it is robust to noise, small gaps in signal, and non-homogeneous signal distribution, such as the signal becoming weaker as it travels away from the injection site. There is also a natural persistence-simplification procedure to help prune less important and reliable branches. We show that our method produce robust data summaries of AAV-tracer injected whole mouse brain data sets from the Mouse Brain Architecture Project. Details of the algorithmic approach together with the resulting skeletonization patterns will be presented.

Disclosures: D.D. Ferrante: None. **S. Wang:** None. **Y. Wang:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.03/NNN48

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

Support: H.N. Mahabala Distinguished Chair in Computational Brain Research, IIT Madras

Title: Automated segmentation of Nissl-stained somata from whole-brain histological image data

Authors: *A. SINGH[1], T. PARAG[2], D. FERRANTE[3], P. MITRA[3];
[1]Electrical engineering, IIT Madras, Chennai, India; [2]HHMI Janelia Res. Campus, Ashburn, VA; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Modern neuroanatomical research relies on whole brain imaging usinglight microscopic techniques. Applications of current interest include mesoscale mapping of connectivity at a whole-brain scale and other structural/functional whole-brain studies (in mouse, marmoset, and other species). An important step of a neuroanatomical study is ”mapping”, ie identifying the brain compartment in which a labelled cell or process is located. Classically, this mapping was done by visual examination of one or more histochemical stains, of which the Nissl stain is the most widely utilized variety, dating from the times of Brodmann. In the modern era of neuroanatomical research, it is desirable to perform this step using techniquesfrom machine vision, given the large data volumes (~ 10^{12} pixels/brain for mouse). As a first step, we have developed algorithms to segment Nissl-stained sections into the component objects (somata of neurons, glial cells and other microscopic objects in the image), which can be further grouped to obtain information about the brain region involved. Several past studies (e.g., Inglis et.al. 2008,

J. Microscopy 230(3):339-52; He et.al. 2015, Scientific Reports 5, no. 12089) addressed similar problems by fitting preconceived shape models to a set of image pixels. Given the large variation in the shapes and pixel intensities of the objects of interest, conceptually it is more reasonable to learn their appearances and boundaries instead. Weadopt such an approach proposed in Parag et.al. (2015, PLoS One, 10:e0125825) for electron microscopy data segmentation. Each pixel of a Nissl image is classified into 3 classes: cell interior, cell boundary and background. On the real valued outputs of a pixel detector, we apply a region growing algorithm (watershed) that typically oversegments the cells into fragments or superpixels. A superpixel agglomeration algorithm, equipped with a superpixel boundary classifier, is then utilized to refine the oversegmentation to produce the final result. We have used interactive inter-faces for training the pixel classifier (ilastik.org) as well as the superpixel boundary classifier (implementation of technique described in Parag et.al., 2014, MICCAI, LNCS 8673:389-397) with limited groundtruth data to generate our baseline segmentation results. On a small set of test images with sparsely located cells, we achieved an F-score of 88% of precision-recall values (at 94% recall) in determining the cell centers computed from the segmentation output. A natural future direction to improve this performance is to train a deep convolutional network on densely annotated images for pixel classification.

Disclosures: **A. Singh:** None. **T. Parag:** None. **D. Ferrante:** None. **P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.04/NNN49

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

Support: Keck Foundation

Mathers Foundation

CSHL Crick-Clay Professorship

H. N. Mahabala Chair (IIT Madras)

NSF EAGER 1450957

NIH MH105971

NIH DA036400

Title: Automated detection of GFP labelled nuclei in whole-brain light-microscopic data sets for mouse with high precision and recall.

Authors: *S. DAS[1], V. V. GOPAL[1], G. PAHARIYA[1], D. D. FERRANTE[2], P. P. MITRA[2]; [1]Computer Sci. and Engg, Indian Inst. of Technol. Madras, Chennai, India; [2]Cold Spring Harbor Lab., New York, NY

Abstract: Whole-brain data sets with light microscope resolution are now available using a variety of light microscopic methods, with applications to structural and functional neuroanatomy. The size of these data sets (~1TB) make it necessary to develop automated methods for detection and counting of objects of interest in the image. We studied the automated detection of GFP labelled nuclei in wide-field fluorescent microscopy, and applied the resulting algorithms to the analysis of the spatial distribution of a set of CRE-defined GABAergic neuronal types in wild type (+/+) and ASD model (16pdf/+) mice.
We found that classical image processing techniques produced effective algorithms with high precision and recall (~96% on average). The algorithms we have developed for these data proceed via detection of salient regions, boundary detection, distance transform of the edge map leading to detection of peaks and ridges, and consequent detection of cell centers. This process efficiently detects the cell centers including the complex case of overlapping cells. The most significant stage is the ridge detection method involving a sequence of six sub-stages.
Manual annotation of two whole mouse brains (~1 Teravoxel each) was done by human operators (a 10 member team cross-verified by two master annotators) to produce the ground truth of cell centers for use in performance evaluation. The average Precision-Recall values for the two brains (totaling ~600 Gigapixel images), was ~96%, indicating the efficiency of the algorithm used. The accuracy of this algorithm was found to be better than recent competitive baseline methods for supervised object detection (ADABOOST, Latent SVM trained using Deformable Part Models). Supervised methods were observed to fail in the case of detecting overlapping cells, as in these cases the foreground blobs form a large variety of non-unique (2D) shape patterns with different sizes. The computational time required to analyze each Gigapixel image, for cell center detection, was around 5 minutes on an i7, 3GHz CPU, 64GB RAM. Output of the automatic cell detection algorithm were used as inputs to subsequent analysis stages (estimation of the spatial density and statistical properties of the centers of the detected nuclei) as well as for visualization purposes. The details of the algorithmic framework and comparison of the results with the comprehensively manually annotated data sets will be presented.

Disclosures: S. Das: None. **V.V. Gopal:** None. **G. Pahariya:** None. **D.D. Ferrante:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

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Program#/Poster#: 470.05/NNN50

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

Support: NSF Eager 1450957

NIH DA036400

NSF CCF-1319406

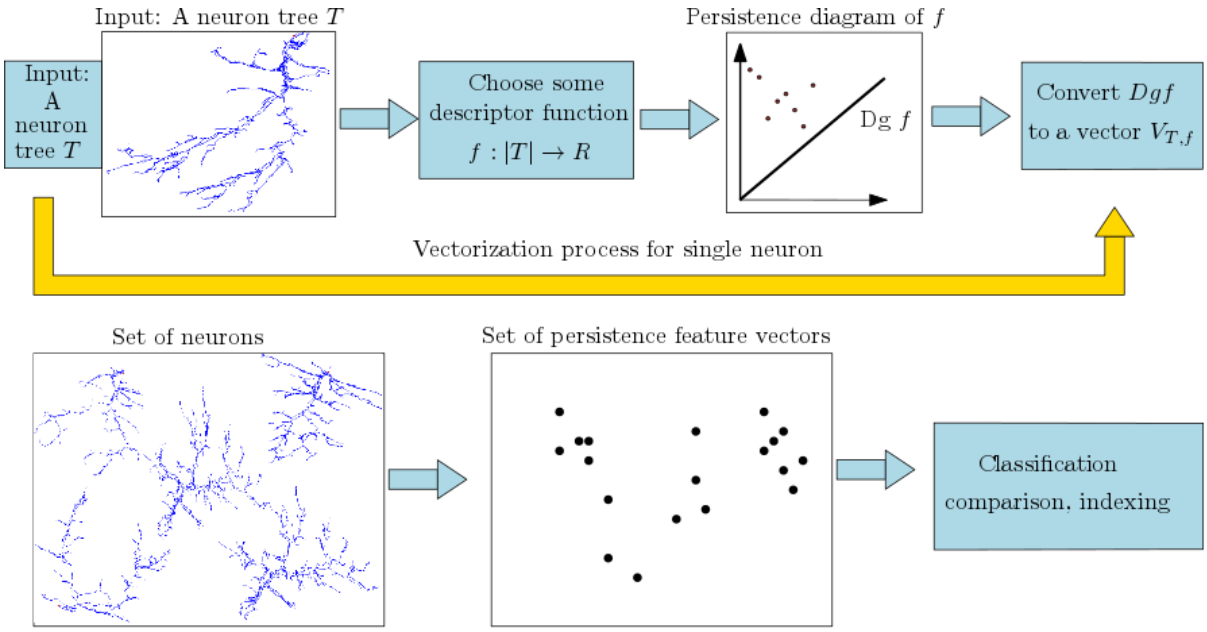
Mathers Foundation

Title: Methods from computational topology for comparing neuronal shapes

Authors: *Y. WANG[1], Y. LI[1], G. A. ASCOLI[2], P. P. MITRA[3];
[1]Computer Sci. and Engin., The Ohio State Univ., Columbus, OH; [2]Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Classifying neuronal shapes is a classical problem in neuroscience with current relevance: high throughput EM and LM based reconstructions are giving rise to large numbers of reconstructed neurons (cf: NeuroMorpho.org, FlyCircuit.org) that call for advanced and automated analysis techniques. Existing approaches mostly convert neuronal shapes into feature vectors, largely manually determined. Once neurons are described as vectors, standard vector-space concepts (eg the Euclidean distance between vectors to classify neuronal shapes) can be used. However, neuronal shapes are fundamentally non-linear objects, which cannot easily be "added" or "subtracted". Therefore it is of interest to look at topological or geometrical analysis methods that respect the non-linear structure of neurons. Such methods have recently gained ground in other areas of data analysis, but are not yet represented in neuronal shape analysis.
In the present work we employ methods from computational topology to define metrics in the space of neuronal shapes, based on ideas of persistent homology. The tree-metrics thus derived do not rely on hand-tuned features, and can be extended to handle developmental dynamics or neuronal biophysics via the incorporation of electronic distances. These considerations lead to a natural feature vectorization based on so-called persistence-diagram summaries of tree shape. Direct metric-space approaches without resorting to intermediate feature-vector descriptions are simultaneously available using Euclidean distance (or other Lp metrics) in the persistence-based feature space.
We successfully apply these methods to classify LM and EM reconstructed neuronal shapes, drawn both from vertebrate and invertebrate preparations, and are able to recover known neuronal classes "out of the box" (ie without hand-tuned features). The methods are scalable

(have controlled computational complexity) and should enable automated analysis of large data sets.



Disclosures: **Y. Wang:** None. **Y. Li:** None. **G.A. Ascoli:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

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Support: NIH TR01 MH087988

NIH RC1 MH088659

The Mathers Foundation

CSHL Crick-Clay Professorship

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NIH DA036400

Title: A comprehensive data set for whole-brain mesoscale connectivity mapping in mouse using injections of a viral anterograde tracer (AAV) on a brain-wide grid

Authors: *A. S. TOLPYGO, D. D. FERRANTE, F. MECHLER, S. SAVOIA, N. FRANCIOTTI, P. P. MITRA;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The architecture of brain connectivity supports and constrains nervous system function and animal behavior and reflects the work of evolution. The Mouse Brain Architecture (MBA) project aims at the comprehensive mapping of brain connectivity at the mesoscopic scale using systematic injections of anterograde and retrograde tracers on a grid of locations covering the mouse brain. We report the completion and release of a dataset of 605 whole mouse brains with 915 injections of anterograde viral tracers comprehensively covering the left hemisphere. A majority of animals were iontophoretically injected [405 animals, 715 sites] with AAV vectors in two colors (AAV2/1.CAG.tdTomato.WPRE.SV40, AAV2/1.CB7.CI.eGFP.WPRE.rBG) at pairs of sites in each brain, sampling the volume of the left hemisphere on an unbiased 3D grid. Pressure injection accounts for 22% of the material, largely for superficial and deep cortex, sampling 200 of 915 sites. Whole brains were coronally sectioned at 20µm thickness producing an alternating series of fluorescent label and Nissl stain using a customized tape-transfer method. Series were imaged at 0.46µm in-plane resolution using the NanoZoomer 2.0-HT system and each ~600 section brain was 3D-reconstructed by rigid registration of sections in each image modality. Successful injections were quantified by computational image analysis for anatomical localization, bolus volume, and the number of infected neuronal somata. The mean distance between injected sites is approximately 335µm, with 75% of all injection centers within 457µm of each other. The estimated interquartile bolus diameters are between 250-550µm with corresponding injection volumes from 0.079 to 1.01 mm^3 dependent on target region and delivery method. In comparison with two projects (AIBS Phase-I, Oh, S.W. et al., doi:10.1038/nature13186; MCP, Zingg, B. et al., doi:10.1016/j.cell.2014.02.023), the MBA dataset has finer section spacing (2.5x and 5x more sections), while providing direct measurements of cytoarchitecture via the alternate Nissl series. The dataset features a higher frequency of injections in the sample volume, with smaller mean distances and similar injection volumes. The MBA dataset and associated metadata are made freely viewable with the aid of a 3D injection browser and a high-resolution image viewer interface at the project website (http://mouse.brainarchitecture.org/). Details regarding the injection grid planning and refinement, data acquisition and quality control, and analysis of the completed AAV data set will be presented.

Disclosures: A.S. Tolpygo: None. **D.D. Ferrante:** None. **F. Mechler:** None. **S. Savoia:** None. **N. Franciotti:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

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Program#/Poster#: 470.07/NNN52

Topic: I.02. Systems Biology and Bioinformatics

Support: HN Mahabala Chair Professorship in Computational Brain Research, IIT Madras

Title: A novel method for extracting brain connectivity information from neuroscience text articles

Authors: A. NAIDU[1], *J. JAYAKUMAR[1], S. CHAKRABORTI[1], A. SHARMA[1], P. SREENIVASA KUMAR[1], D. DEODHARE[2], P. P. MITRA[3];
[1]Computer Sci. and Engin., Indian Insitute of Technology-Madras, Chennai, India; [2]Ctr. for Artificial Intelligence & Robotics (CAIR), DRDO, Bangalore, India; [3]Cold Spring Harbor Labs., Cold Sping Harbor, NY

Abstract: Mapping brain connectivity is of current interest to neuroscientists. There exists a century of knowledge about neuroanatomical connectivity in the literature. This information is valuable and attempts have been made to manually curate brain connectivity databases (cf. BAMS, Bota et al., 2012 *Front Neuroinform*, 6:2;CoCoMac, Bakker et al., 2012 *Front Neuroinform*, 6:30). However, manual curation is labour intensive, raising the need for automated extraction of connectivity information using Natural Language Processing techniques to convert them into a structured database of connections (cf. Richardet et al., 2015 *Bioinformatics*, 31:1640).
In this study, we provide a solution that views the problem of identifying connected brain regions from natural language sentences as that of extracting the relation *"isConnectedTo"* between a given pair of brain region mentions. Traditionally, relation extraction is done either using supervised learning methods that require considerable amount of labelled data or rule based approaches, where rules for extracting connectivity patterns are manually handcrafted. Our approach uses semi supervised learning that significantly lowers human intervention.
For the first part, we have used a surface learning approach and explored feature representations using the Bag of All Words features and the Connectivity word features (only words that indicate connectivity). We then compared their performance on a benchmark WhiteText dataset (cf. French et al., 2015 *Front Neuroinform*, 9:13).
Experiments on the dataset show that our approach using the Bag of All Words features scores well in recall (84%) but with low precision (31%), which can be attributed to the feature representations that include words that are quite noisy to determine connectivity relations.
Our connectivity word feature approach, derived with the help of domain experts, has a reasonably high precision (64%) but poor recall (52%), which can be explained by connectivity

descriptions that our feature words could not capture.
In the second part of our study, we have used syntactic learning using linkages between words by parsing the sentences and representing the shortest path between two brain regions within the link parse as a series of edges and the corresponding words that these edges connect. This approach gives the same recall (84%) but better precision (39%) than the Bag of All Words. We believe to have developed a text mining algorithm for extracting brain connectivity information that is promising but needs further refinement to improve its precision.

Disclosures: **A. Naidu:** None. **J. Jayakumar:** None. **S. Chakraborti:** None. **A. Sharma:** None. **P. Sreenivasa Kumar:** None. **D. Deodhare:** None. **P.P. Mitra:** None.

Poster

470. Computational Tools for Circuit Mapping

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Program#/Poster#: 470.08/OOO1

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

Support: NIH Grant ULTTR001108

1U54MH091657

Title: Precision mapping of structural connectomes in individual brains

Authors: *B. MCPHERSON[1], C. CAIAFA[1], A. AVENA-KOENIGSBERGER[1], J. CONTRERAS[2], L. SHEN[2], Y.-C. WU[2], J. GONI[3], A. SAYKIN[2], O. SPORNS[1], F. PESTILLI[1]; [1]Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [2]Radiology and Imaging Sci., [3]Engin., Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Diffusion weighted Magnetic Resonance Imaging (dMRI) and subsequent tractography-based modeling allows characterizing and quantifying connectivity and tissue properties of white-matter in living brains. By integrating this fiber-tracking information with a gray matter parcellation, it is possible to model a human connectome as a structural connectivity matrix. Such network-based representation allows for testing a wide number of integration, segregation, and overall communicability features, such as clustering coefficient and small worldness, which have been compared to differences in behavior, cognition, development and aging processes across groups. To date, connectome research has been concerned primarily with identifying principles of brain function and structure by averaging connectomes from multiple subjects. Recently, renewed interest has emerged in developing precision methods to reliably map individual connectomes. Mapping connectomes reliably in single brains would allow

measuring individuality and variability across humans as well as tracking longitudinally connectome variations within individuals in an accurate way. In this work, we introduce methods to map statistically-validated structural connectomes. We report results on the reliability of connectome estimates and network measures. We used two datasets dMRI (Van Essen et al. 2013; Pestilli et al. 2014) and multiple fiber tracking methods (Tournier et al. 2012; Descoteaux et al. 2009; Basser et al. 2000). We built ten connectomes in each individual brain and applied the Linear Fascicle Evaluation (LiFE; (Pestilli et al. 2014) method to validate each connectome. Whole brain connectomes were generated avoiding fiber-counts measures but using virtual lesions (Pestilli et al. 2014) combined with FreeSurfer (Fischl 2012). To estimate the precision of the LiFE-validated connectomes, we measured the reliability of standard brain network-properties such as clustering coefficient and small worldness across repeated instances of connectomes build in the same individual using each tracking method. We show the degree to which LiFE-based connectomes achieve reliable levels of replicability within repeats of individual subjects while maintaining good levels of between-subject discriminability as observed by within- and between-group variability. Our results show that structural connectomes processed with LiFE offer more reliable estimates of connections between brain regions. The method contributes to precision connectome mapping methods with the potential to impact detection during prodromal stages of disease or for individuals at genetic risk of disease.

Disclosures: **B. McPherson:** None. **C. Caiafa:** None. **A. Avena-Koenigsberger:** None. **J. Contreras:** None. **L. Shen:** None. **Y. Wu:** None. **J. Goni:** None. **A. Saykin:** None. **O. Sporns:** None. **F. Pestilli:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

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Program#/Poster#: 470.09/OOO2

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

Support: Tata Trusts

JC Bose National Fellowship

DST Centre for Mathematical Biology

UGC Centre for Advanced Studies

Title: New methods for obtaining sparse brain connectivity networks

Authors: *G. RANGARAJAN[1], S. MODY[2];
[2]Dept. of Mathematics, [1]Indian Inst. of Sci., Bangalore, India

Abstract: It is well known that large functional networks such as the whole brain networks are often sparse. However, standard functional/effective connectivity analysis techniques often result in dense networks where it is difficult to gauge which interactions are truly significant. We present two methods based on vector autoregressive models that yield highly sparse connectivity maps even when thousands of voxels/channels are analyzed. One of the methods is a bottom up greedy algorithm and the other method follows a top down approach. Applying these methods to publicly available fMRI resting state data, we are able to determine distinct brain network communities that are consistent with known functional regions of the human brain.

Disclosures: G. Rangarajan: None. **S. Mody:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.10/OOO3

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

Support: Natural Science and Engineering Research Council of Canada postdoctoral fellowship

Swiss National Science Foundation #310030-156874, NCCR-Synapsy 51AU40_125759

Center for Biomedical Imaging (CIBM) of the Geneva-Lausanne Universities and EPFL

Leenaards Foundation

Louis-Jeantet Foundation

National Institute of Health 1R01 MH108467-01

J.S. McDonnell Foundation

Title: Path ensembles and a tradeoff between communication efficiency and resilience in the human connectome

Authors: *A. I. AVENA KOENIGSBERGER[1], B. MISIC[2], R. X. D. HAWKINS[3], A. GRIFFA[4], P. HAGMANN[4], J. GOÑI[5], O. SPORNS[2];

[2]Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN; [3]Stanford Univ., Stanford, CA; [4]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland; [5]Sch. of Industrial Engin. and Weldon Sch. of Biomed. Engin., Purdue Univ., West Lafayette, IN

Abstract: Computational analysis of communication efficiency of brain networks often relies on graph-theoretic measures based on the shortest paths between network nodes. Here, we explore a communication scheme that relaxes the assumption that information travels exclusively through optimally short paths. The scheme assumes that communication between a pair of brain regions may take place through a path ensemble comprising the k-shortest paths between those regions. To explore this approach, we map path ensembles in a set of anatomical brain networks derived from diffusion imaging and tractography. We show that while considering optimally short paths excludes a significant fraction of network connections from participating in communication, considering k-shortest path ensembles allows all connections in the network to contribute. Path ensembles enable us to assess the resilience of communication pathways between brain regions, by measuring the number of alternative, disjoint paths within the ensemble, and to compare generalized measures of path length and betweenness centrality to those that result when considering only the single shortest path between node pairs. Furthermore, we find a significant correlation, indicative of a trade-off, between communication efficiency and resilience of communication pathways in structural brain networks. Finally, we use k-shortest path ensembles to demonstrate hemispherical lateralization of efficiency and resilience.

Disclosures: **A.I. Avena Koenigsberger:** None. **B. Misic:** None. **R.X.D. Hawkins:** None. **A. Griffa:** None. **P. Hagmann:** None. **J. Goñi:** None. **O. Sporns:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.11/OOO4

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

Support: SFB 936, Projects C1/C2/B6

Title: Modeling of large-scale functional brain networks based on structural connectivity from DTI: comparison with EEG derived phase coupling networks and evaluation of alternative methods along the modeling path.

Authors: ***M. BÖNSTRUP**[1], H. FINGER[3], B. CHENG[1], A. MESSÉ[2], C. HILGETAG[2], G. THOMALLA[1], C. GERLOFF[1], P. KÖNIG[3];

[1]Dept. of Neurol., [2]Dept. of Computat. Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Inst. of Cognitive Sci., Univ. of Osnabrück, Osnabrück, Germany

Abstract: Aim: The overall aim of the present study is to find out if networks emerging from phase coupling of band-limited oscillatory signals relate to underlying structural connectivity and if computational models can be used to increase the relation. Then, we use our modelling framework to systematically compare several alternative methods along the modeling path in order to assess their impact on the overall fit between simulations and empirical data.
Method: We use DTI data to estimate structural connectivity and subsequently model phase couplings from alpha band-limited oscillatory signals derived from multichannel EEG data. In a second step, we explore several technical alternatives along the modeling path as structural connectivity preprocessing, complexity of the model, source reconstruction and functional connectivity metrics.
Results: Our results show that about 23.4 % of the variance in empirical networks of resting-state fast oscillations is explained by the underlying white matter architecture. By simulating functional connectivity using a simple reference model, the match between simulated and empirical functional connectivity further increases to 45.4 %. Exploration of technical alternatives along the modeling path revealed the following:
First, we find that an augmentation of homotopic connections in the structural connectivity matrix improves the link to functional connectivity while a correction for fiber distance slightly decreases the performance of the model. Second, a more complex computational model based on Kuramoto oscillators leads to a slight improvement of the model fit. Third, we show that the comparison of modeled and empirical functional connectivity at source level is much more specific for the underlying structural connectivity. However, different source reconstruction algorithms gave comparable results. Of note, as the fourth finding, the model fit was much better if zero-phase lag components were preserved in the empirical functional connectome, indicating a considerable amount of functionally relevant synchrony taking place with near zero or zero-phase lag.
The combination of the best performing alternatives at each stage in the pipeline results in a model that explains 54.4 % of the variance in the empirical EEG functional connectivity.
Conclusion: Our study shows that large-scale brain circuits of fast neural network synchrony strongly rely upon the structural connectome and simple computational models of neural activity can explain missing links in the structure-function relationship. Our results serve as a technical orienting frame for the emerging field of brain network modeling.

Disclosures: M. Bönstrup: None. **H. Finger:** None. **B. Cheng:** None. **A. Messé:** None. **C. Hilgetag:** None. **G. Thomalla:** None. **C. Gerloff:** None. **P. König:** None.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.12/OOO5

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

Title: Synthetic connectomics: analyzing evolved neural networks for simple reaching with a tool

Authors: *Y. CHOE, Q. LI, J. YOO;
Texas A&M Univ., College Station, TX

Abstract: Connectomics is proving to be a powerful technique in neuroscience, however, the data is incomplete, and we lack tools for the analysis even if full data becomes available in the near future. Here, we propose a synthetic connectomics approach, where neural circuits evolved in a computer simulation (NEAT algorithm that evolves network topology) is presented as a temporary alternative to the full biological connectome, to facilitate the development of analysis techniques. The main advantage of this approach is that experimenters can have full access (including lesioning and stimulation) to structural, functional, and also behavioral data. In this abstract, we present our work on applying NEAT to control a jointed, articulated limb that can pick up and use a tool to reach targets (Fig 1A-B). It turns out that it is non-trivial to analyze a tiny network of 15 simulated neurons that evolved to solve the task (Fig 1D, hexagons), even with full information from the simulation (Fig 1F: behavior; Fig 1G: neural activity). We tested (1) graph analysis (Fig 1C) where we showed that evolved circuits with more cycles (loops) in their network topology show higher performance, (2) neural activity time series clustering (Fig 1E) where we discovered four main clusters (also marked in Fig 1G, right margin), and (3) aligning behavioral events (Fig 1F, marked as circled numbers) to neural activity (Fig 1G, see bottom row). Our work shows the complexity of connectomic analysis even with tiny (synthetic) neural networks where the full structural and functional data are known, and at the same time the potential utility of a synthetic approach to connectomics.

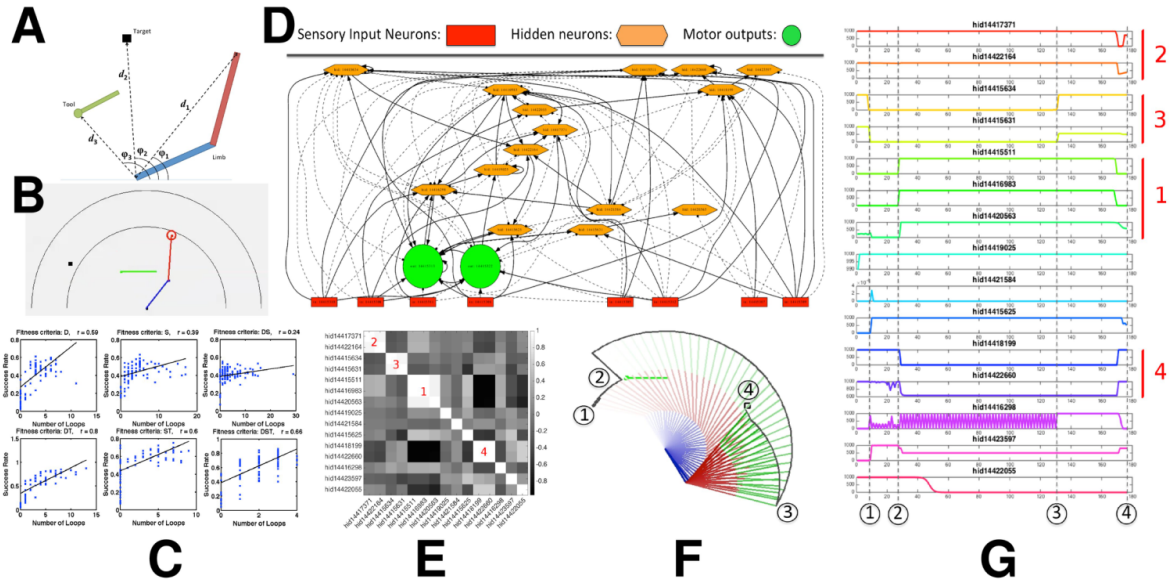

Figure 1. Evolved neural circuit for the control of an articulated arm in a tool-use task for reaching. The controller network receives as input the angle and distance to the target and the tool handle, and outputs the angle of the two joints. See [1] for details.

References 1. Li, Q., Yoo, J., and Choe, Y. (2015). Emergence of tool use in an articulated limb controlled by evolved neural circuits. In Neural Networks (IJCNN), 2015 International Joint Conference on. IEEE.

Disclosures: Y. Choe: None. **Q. Li:** None. **J. Yoo:** None.

Poster

470. Computational Tools for Circuit Mapping

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Program#/Poster#: 470.13/OOO6

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

Support: Sandia LDRD 151345

Title: N2A: a language and software tool for large-scale modeling

Authors: *F. ROTHGANGER;
Sandia Natl. Labs., Albuquerque, NM

Abstract: Sharing models and data is doubtless a prerequisite for progress toward a full understanding of brain function. The Neuroscience Information Framework (NIF) does this for many forms of descriptive data. Ongoing work on interchange languages such as NeuroML/LEMS promises to do this for models. Repositories such as ModelDB hold models in various formats. Excellent graphical tools such as NeuroConstruct support intuitive model building.
However, simply sharing models and data is not sufficient. It is also necessary to assemble those shared models into larger functional units, ultimately reaching the level of an entire nervous system. Beyond that, it is necessary to abstract out algorithmic structures hiding within the details, and to validate models against data.
At higher levels of integration/abstraction, it is necessary to work with declarative models, that is, models which make statements about the attributes of a neural component rather than giving step-by-step instructions for simulating it. Such models are amenable to object-oriented recombination, translation to a wide range of simulators, and algorithmic analysis.
We devised the N2A language [Rothganger et al., Frontiers in Neuroscience 2014] to explore these large-scale modeling concepts. It is a work in progress. We will demonstrate an open-source implementation which combines models and translates them to run on several target simulators, including generated C++ code.

Disclosures: **F. Rothganger:** A. Employment/Salary (full or part-time): Sandia National Laboratories.

Poster

470. Computational Tools for Circuit Mapping

Location: Halls B-H

Time: Monday, November 14, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 470.14/OOO7

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

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Defense Advanced Research Project Agency and the U.S. Army Research Officer Grant W911NF-10-1-0059

Title: Identifying interactions in neural circuit simulations and other data using non-linear multi-dimensional hidden-state models

Authors: ***L. G. GIBB**[1], A. FRIEDMAN[1], J. F. SLOCUM[1], D. TYULMANKOV[1], A. ALTSHULER[2], S. RUANGWISES[1], Q. SHI[1], S. E. TORO ARANA[1], D. W. BECK[1], J. E. C. SHOLES[3], A. M. GRAYBIEL[1];

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Abstract: Two key challenges in neuroscience are quantifying the strength of non-linear interactions between network nodes (e.g., neurons) and decoding complex network activity. In our companion abstract (Friedman et al. 2016), we introduce our non-linear multi-dimensional hidden state (NMHS) approach, which combines decoding of activity in parallel data streams and computation of the strength of the non-linear interactions among them. Here, we test this approach on computer simulations of non-linear neural circuits, voices of musical groups, and socioeconomic data, comparing the performance of NMHS with that of Granger causality (GC) and cross-correlation (CC). We show that NMHS matches or outperforms GC and CC in many cases. We simulated five distinct two- and three-neuron microcircuits using non-linear Hodgkin-Huxley model neurons coupled via conductance-based models of excitatory and inhibitory synaptic connections. The strength of interactions between model neurons determined by NMHS was correlated strongly with the simulated synaptic strength. By contrast, GC and CC performed relatively poorly in evaluating these non-linear interactions. We investigated the effect of unobserved network nodes on the ability of NMHS to estimate the strength of interaction between observed nodes. In one set of simulations, we varied the number of unobserved model presynaptic neurons making synaptic connections onto the observed postsynaptic neuron. In a second set of simulations, we varied the strength of the synaptic connection from a single unobserved model presynaptic neuron onto the observed postsynaptic neuron. As expected, the calculated strength of interaction between observed neurons decreased as the number or synaptic strength of unobserved inputs increased. As a further validation of the method, we used NMHS to analyze interactions among three to six guitarists improvising classic rock music. One musician was a leader and the others were followers in determining the rhythm of the music. NMHS successfully found a greater total interactivity among co-improvising musicians as contrasted with separately improvising musicians or randomly shuffled recordings, and also found interactions between musicians that GC was unable to detect. In a final test, we applied NMHS to three sociodemographic datasets and found that it successfully identified interactions between income and education level. Our work suggests that the NMHS approach is a powerful tool in analyzing neural circuits and other multi-dimensional, non-linear networks.

Disclosures: **L.G. Gibb:** None. **A. Friedman:** None. **J.F. Slocum:** None. **D. Tyulmankov:** None. **A. Altshuler:** None. **S. Ruangwises:** None. **Q. Shi:** None. **S.E. Toro Arana:** None. **D.W. Beck:** None. **J.E.C. Sholes:** None. **A.M. Graybiel:** None.

Poster

470. Computational Tools for Circuit Mapping

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Title: Non-linear multi-dimensional hidden state models for the analysis of neural circuits

Authors: *A. FRIEDMAN[1], J. F. SLOCUM[1], D. TYULMANKOV[1], L. G. GIBB[1], A. ALTSHULER[2], S. RUANGWISES[1], Q. SHI[1], S. E. TORO ARANA[1], D. W. BECK[1,3], J. E. C. SHOLES[3], A. M. GRAYBIEL[1];
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Abstract: There is a critical need in neuroscience and other fields to find connections between nodes with non-linear interactions and to decode the activity of complex networks. Currently, no mathematical or computational approaches can reliably find non-linear interactions in multi-nodal networks and simultaneously decode network activity. Granger causality (GC) and cross-correlation (CC) are leading methods for computing the strength of interactions among nodes, but they are not well suited for the analysis of non-linear interactions. Hidden Markov model (HMM) approaches decode the activity of single nodes but do not incorporate information about other nodes in the network. Here, we introduce a non-linear multi-dimensional hidden state (NMHS) approach, which is a generalization of the HMM permitting simultaneous decoding of activity from multiple parallel data streams and calculation of the strength of the interactions among them. In this approach, the distribution of possible outputs given that the model is in a specific state is summarized by an emission matrix, and the probability of transitioning to each possible state is summarized by a transition matrix. An optimization algorithm finds a model that best accounts for the data. The interaction strength between two nodes is defined as the mean change in the transition matrix of one when the other changes its state. We applied this method to putative microcircuits of two or three neurons in the prefrontal cortex, dorsomedial striatum and substantia nigra pars compacta of rats recorded using tetrodes. Putative bidirectional or unidirectional connections in such microcircuits were determined using antidromic and orthodromic microstimulation, and the neurons were recorded while rats performed a decision-making task in a T-maze. Connections in two-neuron microcircuits were identified correctly by NMHS, GC, and CC. However, strikingly, NMHS was more successful than GC and CC in

analyzing three-neuron microcircuits, in which GC and CC often erroneously found interactions between neurons recorded in different sessions. NMHS also excelled in decoding when compared with HMM: the hidden states that it identified in a three-neuron microcircuit better reflected the rats' behavioral states in the decision-making task. Thus, our novel approach for network analysis compares favorably to GC and CC in detection of interactions, and it also has a substantial advantage in network decoding. Our successful tests of this approach on computer simulations of non-linear neural circuits, voices of musical groups, and socioeconomic data are presented in a companion abstract (Gibb et al. 2016).

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Poster

470. Computational Tools for Circuit Mapping

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Program#/Poster#: 470.16/OOO9

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

Title: What do neurons do? A similarity matching perspective

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Abstract: Our brains analyze high-dimensional stimuli streamed by our sensory organs in multiple stages. Sensory cortices, for example, perform tasks like dimensionality reduction, sparse feature discovery and clustering. To model these unsupervised learning tasks, we pursue a principled approach and propose a new family of objective functions based on the principle of similarity matching. The principle of similarity matching proposes that similar inputs to a neural circuit should evoke similar outputs. We show that many unsupervised learning tasks can be formulated as solutions to similarity matching cost functions under different constraints. From these objective functions we derive online distributed algorithms that can be implemented by biological neural networks resembling cortical circuits. Our networks can adapt to changes in the number of latent dimensions or the number of clusters in stimuli. Furthermore, we extend our similarity matching framework to minimax optimization problems from which we derive online algorithms with two classes of neurons identified with principal neurons and interneurons. Our analysis suggest that the role of interneurons may be to clamp the power dimensions of principal neuron activity. We test our theory using recordings from various brain areas.

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Poster

470. Computational Tools for Circuit Mapping

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

Support: NIH Grant R01 DC009977 from NIDCD

Title: Development of FunctionalConnectomeDB within SenseLab to incorporate and mine functional connectomics data

Authors: ***L. MARENCO**[1,2,4], R. WANG[1], R. A. MCDOUGAL[2], T. M. MORSE[2], N. T. CARNEVALE[2], P. L. MILLER[1,4,3], G. M. SHEPHERD[2];
[1]Med. Informatics, [2]Neurosci., [3]Anesthesiol., Yale Univ. Sch. of Med., New Haven, CT; [4]VA Connecticut Healthcare Syst., West Haven, CT

Abstract: In order to foster discovery, in recent years the SenseLab group has enhanced neuronal content by incorporating functional connectivity information. This information, often referred to as connectomics, is fueled by research on fiber tracts and interconnectivity within brain regions. To overcome the difficulties posed by mining the highly interdisciplinary content within connectomics data, SenseLab has created several techniques to share, navigate, query and represent this information. For data sharing, we use domain-specific data models that are exposed using common internet formats such as JSON. For querying and display we have created innovative realistic microconnectome-enhanced diagrams, as well as continued to develop metadata-driven canonical diagrams. All our components (data, messaging, and client tools) are built using separation of concern principles, as independent tools that can be used separately one from each other, and with similar components from other projects.SenseLab, as an interoperable suite of databases, also supports research on dendritic properties and synaptic organization. Building on the SenseLab extensible data model, we have successfully extended our domain to incorporate the new connectivity details at the neuronal compartment level. Previously we described this information in NeuronDB, but challenges by the complexity of the information, and how to query and navigate it, required the creation of a new database, FunctionalConnectomeDB. This new resource for connectomics data in SenseLab, besides providing connectivity information, also integrates its information with that in our other neuronal databases.Current categories of information in FunctionalConnectomeDB include: a) Microcircuit; b) Brain Regions, c) Neuron: type (principal and interneurons), canonical form and compartments, and relative size; and d) Synapses: transmitter type released from a cell

compartment (e.g. glutamate from an axon terminal) and receptor type activated from a cell compartment (e.g. NMDA receptor in a proximal dendrite).
For more information about FunctionalConnectomeDB, follow http://ycmi.med.yale.edu/PubLinks/HBP/2016SfNFunctionalConnectomeDB

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Poster

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

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Title: Neural decoding of motor responses with Bayesian graphical models

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Abstract: Neural decoding models are most often used to reconstruct a stimulus from neural data from sensory encoding areas. If the neural data come from a motor planning area, decoding principles allow one to instead predict a motor response from the neural data. A wide variety of neural decoding methods exist (e.g., population vector decoding, machine learning methods), however, the focus of these methods is typically restricted to predictive accuracy. This is because these methods are general. We introduce a custom decoder implemented as a Bayesian graphical model that not only allows for accurate behavioral predictions, but also allows for many parameters with meaningful interpretations (e.g., response bias) to be simultaneously estimated. These parameters may subsequently be used as the basis for further inference.
We showcase the power of this approach through application of the model to a dataset collected by Li, Gerfen, and Svoboda (2014). In their behavioral task, mice reported the position in which a bar had previously appeared with a lick to the left or a lick to the right. During the task, extracellular recordings were taken from the anterior lateral region of motor cortex (ALM). Previous research suggests that the pyramidal neurons in this area encode the upcoming motor response (i.e., the licking movement). We first used the custom Bayesian decoder to validate this assertion. If ALM neurons encode the upcoming motor response, then the model should be able

to predict lick direction from the neural data alone. Because lick responses were predicted with ~80% accuracy (chance = 50%; 50/50 split of trials between train and test), we conclude that the population of pyramidal ALM neurons indeed does encode upcoming motor responses.
We then used the Bayesian decoder to validate Li and colleagues' finding that the great majority of individual ALM neurons exhibit significantly different firing rates in advance of different motor responses. To test this, we examined the posterior distributions of all response preference parameters, which quantify the selectivity of each neuron for an upcoming left or right response. This analysis, which would not be possible with most traditional decoding methods, showed that a smaller proportion of ALM neurons are individually selective for the upcoming motor response than was previously thought.
We discuss how this novel decoder may be easily altered to accommodate different experimental contexts, and thus may be viewed as a generalizable approach to neural decoding in a Bayesian framework.

Disclosures: **B. Baribault:** None. **J. Vandekerckhove:** None.

Poster

470. Computational Tools for Circuit Mapping

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

Title: Towards optimal information storage in hierarchical neural circuits

Authors: *A. ALEMI;
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Abstract: A main challenge in neuroscience is finding a general computational principle that explains why cortical circuits are organized in particular structures. Some pioneering works have applied the principle of optimal information storage to successfully make prediction about neural circuit architectures (Brunel 2004, 2016). However the application of this principle to hierarchical neural circuits is poorly understood. Here, I propose a hierarchical attractor network that can achieve an ultra high information capacity. The network has two layers: a visible layer with Nv neurons, and a hidden layer with Nh neurons. The visible-to-hidden connections are set at random and kept fixed during the training phase, in which the memory patterns are stored as fixed-points of the network dynamics. The hidden-to-visible connections, initially normally distributed, are learned via a local, online learning rule called the Three-Threshold Learning Rule, which does not rely on explicit error signal for learning. There are no within-layer connections. Random, uncorrelated patterns were stored at the dense regime in a network of

binary units. The simulation of the full-network suggests that capacity at zero robustness against noise grows exponentially with the expansion ratio Nh/Nv. Preliminary analytical calculation of capacity for a mean-field approximation of the model provides a lower-bound for such increase. Increasing robustness reduces drastically the high storage capacity, suggesting that single-layer of random projection amplifies the noise in the visible layer. Increasing the number of hidden layers and making the representation sparse may help to keep the high-capacity with a finite robustness (providing generalization). Additionally, it was observed that, at maximal capacity, the degree of symmetry of the connectivity between the hidden and the visible neurons increases with the expansion ratio. In summary, this work suggests that expansive hierarchy in neural circuits provides a computational advantage in terms of information storage.

Disclosures: A. Alemi: None.

Poster

470. Computational Tools for Circuit Mapping

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

Support: NSF IIS-1302125

Title: Replicating neurophysiological data with spiking neural networks utilizing an evolutionary framework

Authors: *E. ROUNDS[1], A. ALEXANDER[2], E. SCOTT[3], K. DE JONG[3], D. NITZ[2], J. KRICHMAR[1];
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Abstract: We introduce a framework to efficiently match simulated neural networks to neurophysiological datasets recorded from awake, behaving animals. In this work, we use spiking neural networks (SNNs), which are increasingly relevant tools for the computational modeling of cognitive function as high-performance computing resources become available. They allow researchers to formulate models that are capable of capturing important realistic features of neurobiological activity, including spiking dynamics, synaptic conductances, and plasticity while retaining sufficient computational efficiency to enable the construction of large-scale networks. Thus, researchers are able to study the circuitry and mechanisms underlying specific cognitive phenomena using biologically plausible SNNs of neuronal activity and behavior. However, in order for such simulated networks to offer significant explanatory power,

they must link to, and corroborate, experimental data at multiple levels. An important step in this process involves verifying SNNs by using them to reproduce electrophysiologically recorded neuronal firing rates, but as models of brain function increase in size and complexity, it also becomes intractable to manually tune the increasingly large number of open parameters. To address these challenges, we use a GPU-accelerated automated tuning framework employing an evolutionary algorithm to optimize the synaptic plasticity parameters of a SNN in order to match synthetic neuronal firing rates with experimentally observed firing rates. The framework was applied to a neurophysiological dataset recorded from the rodent retrosplenial cortex (RSC) in which four relevant recorded behavioral variables were provided as inputs to each SNN (head direction, linear and angular velocity, and allocentric position) and then correlating the synthetic firing rates with the experimentally observed firing rates. We found that our evolved SNNs captured the behavior of the dataset at the level of the neuronal recordings (e.g., firing rates and patterns), the functional aspects of the neuronal population (e.g., turn, route, and place specific responses), and behavioral features of the data at the population level (e.g., route reconstruction). Thus, our framework produced networks whose behavior transcended multiple levels of the recorded data. This framework may be extendable to other neurophysiological datasets recorded under similar conditions in conjunction with the appropriate behavioral inputs.

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Poster

470. Computational Tools for Circuit Mapping

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

Title: Multiscale interactions predict stress in adult zebrafish (Danio rerio).

Authors: *K. M. KHAN, E. M. CARAMILLO, A. D. COLLIER, J. K. DOYON, J. D. CLARK, T. SURBER, A. HAJNAL, D. J. ECHEVARRIA;
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Abstract: Zebrafish are a well-established translational model in neurobehavioral sciences. Several behavioral paradigms have been translated from rodent tasks to the zebrafish, as this aquatic model is amenable to high-throughput screening. In an effort to automate and streamline phenotypic screening in zebrafish, several research groups have implemented the use of video-aided computer tracking software in their research. In the present study, we compare the use of traditional behavior tracking procedures against non-traditional techniques. Adult zebrafish

exposed to a stress procedure (15 min isolation and confinement) were transferred to an open field task to assess stress-like behavior. Swim behavior was analyzed with computer tracking software (idTracker, Madrid, Spain) and video differencing in ImageJ (NIH, Bethesda, MD). Video differencing compares average pixel intensity in neighboring frames and is used as a measure of temporal change in human coordination tasks. Both resulting time series were analyzed using multifractal detrended fluctuation analysis (MFDFA). The results indicate that the traditional and non-traditional methods are equivalent, and equally capable of predicting stress conditions and behavioral outcomes. Further, multi-fractality contributes to the prediction of stress response above and beyond traditional measures of variance, specifically standard deviation. This work highlights the novel concept of predicting internal states based on structure of variability in swim patterns in adult zebrafish. Implications for developing novel procedures for diagnosing stress based on monitoring multiscale interactions in motor behavior are considered.

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Poster

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Title: Future-proof digital representation of neuronal morphologies

Authors: *B. TORBEN-NIELSEN[1], E. BAS[2], W. CHEN[3], H. CUNTZ[4,5], J. KIM[6], Y. KUBOTA[7], A. M. MOORE[8], C.-T. SHIH[9], G. TAVOSANIS[10], H. PENG[11], G. A. ASCOLI[12], E. DE SCHUTTER[3];
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Abstract: Recent advances in biological imaging technologies allow us to investigate neuronal morphologies at a level unthinkable a decade ago. With light microscopy and modern day virus-based labelling techniques, different markers can be visualized simultaneously and growth can be traced. Electron microscopy meticulously outlines neuronal structures at the cellular and subcellular level. When imaging larger regions, all neurons and their interconnections can be tracked. With time, more data with higher spatial and temporal resolution over ever expanding regions will become available. How will we process all this data into meaningful insights? The answer involves collaboration, sharing and automation of work-flows, and big-data on high-performance computing platforms [1].
However, prior to addressing this question, the available data need to be stored unambiguously to unleash the full potential of big data for most effective sharing and greatest scientific impact. The current de facto standard to digitally store neuronal morphologies is the SWC format [2]. This format is two decades old and represents individual neurons as a set of linked cylinders. Its simplicity is its virtue: detailed morphologies can be represented in a parsimonious way. However, current day neuronal imaging techniques provides far more detail than can be stored. As secondary use of morphological data becomes wider practice [3], we aim to store as much detail as the experimentalist is willing to store; a limitation imposed by the format itself is unacceptable.
We propose an extension of the SWC format to include time-resolved morphological data, contextual data (such as generic environmental descriptors), and subcellular markers. The extended format is implemented as an HDF5 file [4], a binary file format that hierarchically stores data in a way similar to how folders are stored on a hard drive. As such, our proposed format serves as a container storing data related to all aspects of a neuronal morphology. The modular nature of HDF5 files also makes the format future-proof as distinct types of data associated to neuronal morphologies and their biological context can be appended to existing files without losing data. Reasonable backward compatibility with the original SWC format is achieved by also storing SWC data in the new format. Various databases and experimental and analysis tools provide support for this extended format.
[1] Peng et al., Neuron 87:252:256, 2015

[2] Cannon et al., J. Neurosci. Methods 84:49-54, 1998
[3] Ascoli, PloS Comp Biol 13(10): e1002275, 2015
[4] http://www.hdfgroup.org/HDF5/

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Poster

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Title: Generating and predicting long-range neural connectivity using the reverse geometric principle

Authors: ***P. H. TIESINGA**[1], M. BAKKER[2], R. BAKKER[3];
[2]Neuroinformatics, [3]Neuroinformatics/Donders Inst., [1]Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Cognitive processes are mediated by spatiotemporally correlated neural activity patterns, whose properties are in part determined by the underlying structural connectivity of the brain. Realistic models therefore need to get connectivity at the cell-to-cell level right. It is currently impossible to measure the connectivity with sufficient resolution across a large enough volume. State-of-the-art approaches therefore predict connectivity based on geometric or statistical principles and validate these using the limited data available. Most of the former approaches are based on Peters' principle, starting from populations of reconstructed morphologies, with close apposition between axons and dendrites predicting the presence of synapses.
Recent work has applied two strategies. First, oversampling synapses followed by pruning based on the number of expected synapses and their multiplicity distribution (the number of synapses between same pair of neurons). Second, by assigning densities to larger volumes, and matching pre and postsynaptic elements. Recent analysis of saturated reconstructions based on electronmicroscopy data has cast doubt on the validity of a direct application of Peters' principle, although it is likely to be valid at the aggregate level.

We introduce a different approach which makes it possible to get the appropriate number of synapses without pruning and incorporate statistical factors in addition to purely geometrical ones. We first place neuron somas according to the reported densities and model their axons and dendrites by smooth parametrized densities that match reconstructed morphologies, and whose parameters are sampled from a distribution representing observed morphological variability. Subsequently, pre and postsynaptic elements are sampled in voxels according to these spatial densities; a colocalization of a pre and postsynaptic element in the same voxel then yields a synapse. From the sampled elements we reconstruct neuronal morphologies and validate these using the experimentally reconstructed morphologies. The key challenge of the algorithm is overlap: where more than one pre (or post) synaptic element is in the same voxel. Removing these leads to bias in the density. We have developed three strategies dealing with overlap and report that so called selection method leads to the lowest bias and is computationally efficient. The tool will be made publicly available as part of the Human Brain Project

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